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THE DISTRIBUTION AND RELATION OF FIBER POPULATION, LENGTH, BREAKING LOAD, WEIGHT, DIAMETER, AND PERCENTAGE OF THIN-WALLED FIBERS ON THE COTTONSEED IN FIVE VARIETIES OF AMERICAN UPLAND COTTON [1]

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INTRODUCTION

The main object of the work presented in this paper was to determine the distribution and relation of fiber population, length, breaking load, weight, diameter, and percentage of thin-walled fibers on the cottonseed in five varieties of American upland cotton (*Gossypium hirsutum* L.).

Cotton fibers are elongated epidermal cells, or hairs, the cellulose walls of which may show a wide variation in thickness on a single seed of a selected variety of cotton. Fibers on a single seed also usually vary considerably in diameter, length, strength, and unit weight. Since a commercial lot of cotton is derived from many seeds coming from many plants, it is apparent that the variation of any physical fiber property in a commercial sample depends upon the variation on single seeds, between seeds, and between plants. Uniformity in any particular fiber property, such as length, is something which the cotton breeder looks for in making plant selections. If there are inherent differences between plants, the breeder can isolate the plants which show relatively high uniformity for the various physical fiber properties and thus develop superior strains. The success which the breeder may have in making plant selections for fiber properties is limited by the degree of variability of these properties found upon the seeds of a plant. Thus it appears that investigators need much information about the location, the population, and the physical properties of fibers on the seed coat.

Literature on this subject is limited. The author [2] found that the fiber population is sparse at the micropylar end of the seed and becomes denser toward the chalazal region, being densest at the base and around the point where the vascular bundle separates into primary veins. According to Campbell,[3] the percentage of undeveloped fibers (having thin walls) is highest at the basal end of the seed,

[1] Received for publication March 29, 1940. Technical Paper No. 117, Department of Agronomy, North Carolina Agricultural Experiment Station. This paper includes a thesis submitted to Duke University in partial fulfillment of the requirements for the degree of doctor of philosophy, awarded June 5, 1939.

[2] MOORE, JERRY H. A STUDY OF THE DISTRIBUTION OF THE FIBER POPULATION ON THE SEED COAT OF NAKED-SEED COTTON AND OTHER TYPES. 1925. [Unpublished master's thesis. Copy on file N. C. State Col. Libr.]

[3] CAMPBELL, ROY C. SOME FACTORS WHICH INFLUENCE THE DEVELOPMENT OF THE COTTON FIBER CELL WALL. 1926. [Unpublished master's thesis. Copy on file N. C. State Col. Libr.]

becoming lower as the pointed end is approached. He states that this fact, together with the previous research on fiber distribution on the seed coat, indicates that the percentage of undeveloped fibers increases as the density of fiber population increases. Campbell concludes that the percentage of undeveloped fibers on a single seed or plant may be influenced by variation in fiber length, the area of longer fibers being associated with the greater percentage of undeveloped fibers. Koshal and Ahmad [4] studied the fibers on single seeds of Surat and Standard Indian cottons. They report that the mean fiber length for fibers at the base of the seed is significantly greater than for fibers at the apex of the seed; that the mean fiber weight per unit of length and the mean fiber strength are significantly greater for the apical than for the basal fibers; that the values of the mean fiber lengths, fiber-length distribution, fiber weight, and fiber strength are practically the same for the right and the left flanks of the seed coat; and that there is a distinct tendency for a high ginning percentage to be associated with low percentage differences between mean fiber length of hairs taken from the apex and the base of a seed.

MATERIALS AND METHODS

Five improved commercial varieties of American upland cotton bearing a full coat of fuzz hairs on the seed were selected as material for the investigations. Previous measurements on these varieties had indicated that they show differences relative to certain boll, seed, and fiber properties.

The five varieties were grown at Raleigh, N. C., during the 1937 season, with two one-row replications for each variety. The varieties received the same cultural and fertilizer treatment, which was similar to that usually given cotton, and the soil appeared to be uniform. Therefore variation caused by environment was probably not important. The planting plan is given in table 1.

TABLE 1.—*Planting plan of the five cotton varieties grown for the fiber distribution and correlation studies*

Series No.	Variety	Row No. [1]	Series No.	Variety	Row No. [1]
1	Mexican 128	1	2	Mexican 128	6
	Coker-Cleveland 884-4	2		Coker-Cleveland 884-4	7
	Farm Relief No. 1	3		Farm Relief No. 1	8
	Acala 4067	4		Acala 4067	9
	Rowden 40	5		Rowden 40	10

[1] All rows 150 feet long.

When most of the bolls on the cotton plants had matured and opened, one lock was picked from each of 25 plants of each variety and series. These locks (a lock is the seed cotton in a locule) were picked only from those bolls which appeared to be normal in appearance and free of disease and insect injury. The sample for each variety was put into a paper bag, which was then properly labeled and stored in the laboratory.

In the laboratory 5 locks from each series of each variety were picked at random from the bags containing 25 locks, giving a total sample of 10 locks of each variety. One seed from the middle of each

[4] KOSHAL, RAM SARAN, and AHMAD, NAZIR. VARIATIONS IN THE PROPERTIES OF COTTON FIBRE IN RELATION TO ITS POSITION ON THE SURFACE OF THE SEED. Textile Inst. Jour. 23: T211–T266, illus. 1932.

of 10 locks of each variety was carefully removed so as to leave the attached fibers intact. Such a procedure gave a sample of 10 seeds from 10 random plants of each variety.

A leather punch was used to cut out definite areas from the seed coat with the fibers attached. The author [5] had previously used a different method for counting the fiber population. In the earlier investigation the fibers had been pulled from mature seeds taken from bolls preserved in formalin before they opened, the seeds had been dipped for a few seconds in a staining solution, sections had been cut free-hand from six regions on the seed and mounted on a glass slide, and the number of fiber scars (all fiber scars were stained) had been counted by the aid of a microscope. Knowing the area of the microscopic field, the author had then calculated the fiber population per square millimeter of the seed coat.

In the problem presented in this paper, however, it was necessary that all the fibers from each point on the seed coat be counted and also saved for measurement of some of their physical properties. For this reason the leather punch was used in preference to the method just described. A seed was cut transversely into three parts by using a scalpel, and the embryo was removed. Care was observed to avoid cutting or losing fibers from the selected regions on the seed coat. The cutting with the leather punch was done from the inside of the seed coat. When the punch had just about passed through the seed coat, pressure was released and the circular area carefully removed. Six regions, or locations, numbered one to six, were cut from each seed, the area of each region being 3.567 mm.^{2} The location of these six regions is shown in figure 1. Each individual punch sample from each seed was placed in a folded, labeled paper, and all the punch samples for the seed were put into a paper bag marked with the variety and plant number.

Since the unit fiber weight, the fiber length, and the fiber strength are affected by variations in temperature and the moisture content of the air, measurement of these fiber properties was made in a laboratory where a constant temperature of 70° F. and a relative humidity of 65 percent were maintained.

The seed coat and fuzz hairs were removed from each punch sample by use of a fine steel comb and the fingers. The remaining tuft of fibers in the punch sample was then weighed on an assay balance to the nearest one-hundredth of a milligram. For region 1, one-tenth of the total weight of the punch sample was used for counting the fiber population and determining the fiber length, unit weight, strength, diameter, and percentage of thin-walled fibers; for the other regions one-fourth of the weight of the punch sample was used for obtaining the fiber data just mentioned.

The fibers were counted on a black velvet pad by using a pair of fine tweezers. Since the fibers were counted in only a fraction of the punch sample, the total population for region 1 was obtained by multiplying the fractional population by 10, and the total population of the other regions was obtained by multiplying the fractional population by 4.

The average length of the fibers on each of the six regions was obtained by measuring the individual lengths of 20 fibers taken at random from a fractional part of a punch sample, summing the

[5] See footnote 2.

individual lengths, and dividing by the number of fibers. Each fiber was straightened out on a black velvet pad and then measured to the nearest sixteenth of an inch by means of a small rule.

The fiber weight per inch of each of the six regions was determined as follows: The total number of fibers in each region was multiplied by the average fiber length of the region, giving the total number of

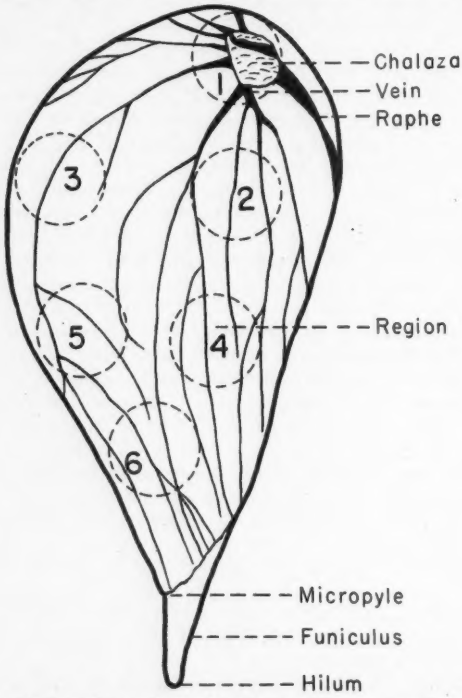

FIGURE 1.—Cottonseed showing the location on the seed coat of the regions selected for studying the distribution and relation of the six fiber characters. Each circle with a dotted line represents a region, or punch area, amounting to 3.567 mm.[2] A plane drawn through the long axis of the seed in such a way as to divide the raphe and regions 1, 3, 5, and 6 into two equal parts would cut the seed into halves. The approximate venation of the seed coat is shown. The fuzz hairs (very short fiber) and the lint hairs (long hairs or commercial fibers) are not shown.

inches of fiber in that region; the total number of inches was then divided into the total fiber weight of the region to obtain the average fiber weight per inch.

The breaking load, or strength,[6] of single fibers was measured upon a testing machine of the balance type. A thin strip of rubber was put on each metal jaw of the testing machine in order to prevent the fiber from slipping and also to avoid crushing the fiber by the jaws. Twenty fibers were taken at random from each region and broken individually. The breaking load was added to the testing beam at

[6] Fiber strength and fiber breaking load mean the same thing in this report; the latter terminology is probably the better.

the rate of one-tenth of a gram per second. The breaking load per fiber was recorded to the nearest one-hundredth of a gram. Three-eighths of an inch at the midportion of the fiber length was exposed to the breaking load. The average breaking load per fiber for each region of each variety was obtained by summing that of 20 single fibers and then dividing the total by 20.

The diameter of fibers on each region was determined from mercerized samples. A small bundle of fibers from each region of the 10 seeds of each variety was mercerized thoroughly in an 18-percent solution of sodium hydroxide and then washed in water and air-dried. The fibers were then placed parallel to one another on a glass slide and mounted in liquid paraffin under a cover slip. By the use of a microprojector apparatus the width of each fiber was measured at the midportion of its length. Twenty fibers from each region on the seed coat were measured, and the average diameter of this number was then calculated.

The 20 fibers which had been mounted for diameter measurements were observed under the polarizing microscope in order to estimate the percentage of thin-walled fibers. The percentage of thin-walled fibers was obtained by observing the kind and intensity of colors and the morphological appearance of each fiber.

EXPERIMENTAL RESULTS

VARIANCE OF FIBER CHARACTERS WITHIN VARIETIES

The distribution of the fiber population in the Mexican 128 variety is given by plants and regions in table 2. Since each plant is represented by only one seed, allowance must be made for the probability that the data from one seed are not necessarily representative for the whole plant. In table 2 plants, or seeds, are designated by the letters A, B, C, etc., whereas the regions on the seed coat are numbered 1, 2, 3, 4, 5, and 6. Fiber population per square millimeter of any mean in table 2 and all other tables for fiber population can be obtained by dividing the mean by 3.567 (the area of each region). Plant mean as used in this paper is the average of the six regions on the seed.

TABLE 2.—*The analysis of variance of the fiber population by plants and regions on the seed coat in the Mexican 128 variety of cotton*

OBSERVED VALUES

Plant	Fiber population for region—						Plant totals	Plant means
	1	2	3	4	5	6		
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
A	2,310	432	500	181	412	404	4,239	707
B	2,070	240	496	148	336	208	3,498	583
C	1,480	200	240	272	307	492	2,991	499
D	492	268	440	808	256	464	2,728	455
E	1,390	488	268	296	276	348	3,066	511
F	1,900	208	392	180	312	328	3,320	553
G	1,720	300	484	216	352	312	3,384	564
H	980	272	1,312	172	424	124	3,284	547
I	1,110	160	296	464	236	248	2,514	419
J	1,120	392	336	124	356	244	2,572	429
Region total	14,572	2,960	4,764	2,861	3,267	3,172	31,596	
Region means	1,457	296	476	286	327	317		

TABLE 2.—*The analysis of variance of the fiber population by plants and regions on the seed coat in the Mexican 128 variety of cotton*—Continued

ANALYSIS OF VARIANCE

Variation due to—	Degrees of freedom	Mean square	*F*			Odds
			Found [1]	Required		
				5 percent point	1 percent point	
Plants	9	43,643	0.51	2.71	4.31	<19:1
Regions	5	2,126,722	24.65	2.42	3.45	>99:1
Error	45	86,260				
Total	59	Standard deviation of the experiment=294				

t TEST

Item	Standard error, any mean	Standard error of the difference between any 2 means	Least difference necessary for odds of—	
			19:1	99:1
Plants	120	170	342	457
Regions	93	132	266	355

[1] 43,643/86,260=0.51, and 2,126,722/86,260=24.65.

Analysis of variance [7] for the data is also given in table 2.[8] It is realized that the groups of data in this table and also in other tables of this paper relative to fiber population and percentage of thin-walled fibers, respectively, may not necessarily fit the homogeneous requirements for the application of variance analysis. However, the individual standard errors for columns or items were calculated and tests made for significance. These tests indicated that interpretation of the results through the use of analysis of variance was satisfactory. The groups of data used for obtaining the values for the analysis of variance in the tables relative to the percentage of thin-walled fibers are analogous to weighted data. The word "percentage" is used in order to avoid the term "immaturity." The results indicate no significant differences between mean populations of plants, since the *F* found is only 0.51 and the *F* value required for odds of 19:1 amounts to 2.71. Odds of greater than 19:1 indicate that differences are significant, and odds greater than 99:1 indicate that differences are highly significant. Between the mean populations of the regions the odds are greater than 99:1, indicating high significance, the required *F* value for odds of 99:1 being 3.45, while the mean square of regions divided by the mean square of the remainder amounts to 24.65, indicating that without doubt the odds are greater than 99:1.

On further analysis, the standard error of any region mean is found to be 93, while the standard error of the difference between any two

[7] FISHER, R. A. STATISTICAL METHODS FOR RESEARCH WORKERS. Ed. 5, rev. and enl., 319 pp., illus. Edinburgh and London. 1934.

[8] The *F* and *t* values for this and all other tables in this paper were obtained from SNEDECOR, GEORGE W. CALCULATION AND INTERPRETATION OF ANALYSIS OF VARIANCE AND COVARIANCE. 96 pp. Ames, Iowa. (Iowa State Col., Div. Indus. Sci. Monog. 1.) 1934. See pp. 88–91.

region means is recorded as 132.[9] Then from t values and the indicated calculations, least differences amounting to 266 fibers for odds of 19:1 and to 355 fibers for odds of 99:1 are found. The mean of region 1 minus the mean of region 3 equals 1,457 minus 476, or 981 fibers. Since 981 is greater than 355, it appears that region 1 certainly has a denser population than region 3 and therefore a denser population than that of any other region. All possible comparisons of regions 2 to 6, inclusive, indicate no significant difference between the means of any pair of these regions.

Since the odds of less than 19:1 in table 2 do not indicate significant differences between plant means, no comparisons for least differences between plants are given.

The fiber length of the Mexican 128 variety is shown by plants and regions in table 3. The plants, or seeds, and the regions are the same as those in table 2, the only difference being that fiber length instead of population is analyzed. The values for the analysis of variance relative to fiber length give odds of more than 99:1 for plant differences and odds less than 19:1 for region differences. As previously described for table 2, least differences can be used to compare any pair of plants or any pair of regions relative to differences between means.

TABLE 3.—*The analysis of variance of the fiber length by plants and regions on the seed coat in the Mexican 128 variety of cotton*

OBSERVED VALUES

Plant	Average fiber length for region No.—						Plant totals	Plant means
	1	2	3	4	5	6		
	Inches	*Inches*	*Inches*	*Inches*	*Inches*	*Inches*	*Inches*	*Inches*
A	1.07	0.99	0.98	0.95	1.09	1.05	6.13	1.022
B	1.08	1.14	1.07	.91	.94	1.08	6.22	1.037
C	1.15	1.18	1.16	1.26	1.23	1.20	7.18	1.197
D	1.00	1.11	1.07	.79	1.00	.96	5.93	.988
E	1.01	1.16	1.00	1.24	1.07	.96	6.44	1.073
F	1.20	1.05	1.05	1.16	1.14	1.05	6.65	1.108
G	1.12	1.19	1.18	1.12	1.15	1.13	6.89	1.148
H	1.26	1.24	1.30	1.25	1.11	1.21	7.37	1.228
I	1.38	1.11	1.30	1.32	1.28	1.21	7.60	1.267
J	1.16	1.07	1.05	.98	1.04	1.07	6.37	1.062
Region total	11.43	11.24	11.16	10.98	11.05	10.92	66.78	
Region means	1.143	1.124	1.116	1.098	1.105	1.092		

ANALYSIS OF VARIANCE

Variation due to—	Degrees of freedom	Mean square	F			Odds
			Found	Required		
				5 percent point	1 percent point	
Plants	9	0.05274	8.56	2.15	2.94	>99:1
Regions	5	.00352	.57	4.36	9.02	<19:1
Error	45	.00616				
Total	59	Standard deviation of experiment=0.0785				

[9] Standard error of any region mean=$294/\sqrt{10}$=93. Standard error of the difference between any two region means=$93\sqrt{2}$=132. The value of t, corresponding to 45 degrees of freedom, the number on which the error is based, is 2.014 at the 5 percent point, and 2.690 at the 1 percent point; then for between region means, least difference at 5 percent point for odds of 19:1=2.014×132=266, and at 1 percent point=2.690×132=355 for odds of 99:1.

TABLE 3.—*The analysis of variance of the fiber length by plants and regions on the seed coat in the Mexican 128 variety of cotton*—Continued

t TEST

Item	Standard error, any mean	Standard error of the difference between any 2 means	Least difference necessary for odds of—	
			19:1	99:1
Plants	0.0320	0.0453	0.0912	0.1219
Regions	.0248	.0351	.0707	.0944

The fiber weight per inch of the Mexican 128 variety is given by plants and regions in table 4. The plants, or seeds, and the regions are identical with those in table 2. The values for the analysis of variance in this table show that the odds for differences in fiber weight are more than 19:1 between plants and greater than 99:1 between regions.

The fiber strength of the Mexican 128 variety is presented in table 5 wherein the values for the analysis of variance indicate real differences in the average fiber breaking load between plants and also between regions.

TABLE 4.—*The analysis of variance of the fiber weight per inch by plants and regions on the seed coat in the Mexican 128 variety of cotton*

OBSERVED VALUES

Plant	Average fiber weight for region No.—						Plant totals	Plant means
	1	2	3	4	5	6		
	0.0001 mg.	*0.0001 mg.*	*0.0001 mg.*	*0.0001 mg.*	*0.0001 mg.*	*0.0001 mg.*	*0.0001 mg.*	*0.0001 mg.*
A	34	44	46	64	49	55	292	49
B	25	55	40	58	54	54	286	48
C	33	51	38	41	46	44	253	42
D	51	67	45	60	65	60	348	58
E	41	43	63	52	65	61	325	54
F	31	62	49	47	49	51	289	48
G	32	36	41	55	43	52	259	43
H	48	59	39	54	50	57	307	51
I	34	75	39	43	55	57	303	51
J	34	50	50	71	54	66	325	54
Region total	363	542	450	545	530	557	2,987	.....
Region means	36	54	45	55	53	56	.....	.....

ANALYSIS OF VARIANCE

Variation due to—	Degrees of freedom	Mean square	*F*			Odds
			Found	Required		
				5 percent point	1 percent point	
Plants	9	145.67	2.46	2.15	2.94	>19:1
Regions	5	583.60	9.87	2.42	3.45	>99:1
Error	45	59.13	.....	.....	.....	.....
Total	59	Standard deviation of the experiment=7.69				

TABLE 4.—*The analysis of variance of the fiber weight per inch by plants and regions on the seed coat in the Mexican 128 variety of cotton*—Continued

t TEST

Item	Standard error, any mean	Standard error of the difference between any 2 means	Least difference necessary for odds of—	
			19:1	99:1
Plants	3.14	4.44	8.94	11.94
Regions	2.43	3.44	6.93	9.25

TABLE 5.—*The analysis of variance of the fiber breaking load by plants and regions on the seed coat in the Mexican 128 variety of cotton*

OBSERVED VALUES

Plant	Average fiber breaking load for region No.—						Plant totals	Plant means
	1	2	3	4	5	6		
	Grams	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
A	4.45	4.75	5.15	6.63	7.45	6.95	35.38	5.90
B	3.35	6.70	4.65	5.30	6.68	6.08	32.76	5.46
C	3.43	5.73	4.48	6.43	6.73	5.80	32.60	5.43
D	3.88	6.93	4.95	4.53	7.35	5.13	32.77	5.46
E	4.23	4.88	5.48	7.65	6.80	7.60	36.64	6.11
F	2.78	6.23	4.40	4.40	5.20	4.35	27.36	4.56
G	3.38	4.08	3.78	6.48	4.68	6.98	29.38	4.90
H	5.55	6.65	5.85	6.50	5.70	7.10	37.35	6.23
I	3.58	4.15	3.90	5.73	5.15	5.63	28.14	4.69
J	4.15	5.23	4.98	7.13	5.68	5.70	32.87	5.48
Region total	38.78	55.33	47.62	60.78	61.42	61.32	325.25	.....
Region means	3.88	5.53	4.76	6.08	6.14	6.13	.....	.....

ANALYSIS OF VARIANCE

Variation due to—	Degrees of freedom	Mean square	*F*			Odds
			Found	Required		
				5 percent point	1 percent point	
Plants	9	1.9333	2.88	2.15	2.94	>19:1
Regions	5	8.5693	12.77	2.42	3.45	>99:1
Error	45	.6711	.....	.....	.....	.....
Total	59	Standard deviation of experiment=0.819				

t TEST

Item	Standard error, any mean	Standard error of the difference between any 2 means	Least difference necessary for odds of—	
			19:1	99:1
Plants	0.334	0.472	0.951	1.270
Regions	.259	.366	.737	.985

The variance analysis for the percentage of thin-walled fibers in Mexican 128, given in table 6, indicates highly significant differences between regions and no differences between plants.

TABLE 6.—*The analysis of variance of the percentage of thin-walled fibers by plants and regions on the seed coat of the Mexican 128 variety of cotton*

OBSERVED VALUES

Plant	Thin-walled fibers for region No.—						Plant totals	Plant means
	1	2	3	4	5	6		
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
A	18	5	20	5	10	17	75	13
B	50	5	20	0	5	0	80	13
C	33	5	20	0	5	0	63	11
D	20	10	9	47	5	5	96	16
E	45	14	0	16	5	5	85	14
F	42	15	27	0	5	0	89	15
G	55	42	35	5	14	0	151	25
H	32	16	60	0	5	10	123	21
I	33	10	24	5	0	5	77	13
J	35	29	20	0	15	5	104	17
Region total	363	151	235	78	64	52	943	
Region means	36	15	24	8	6	5		

ANALYSIS OF VARIANCE

Variation due to—	Degrees of freedom	Mean square	*F*			Odds
			Found	Required		
				5 percent point	1 percent point	
Plants	9	113.00	0.81	2.71	4.31	<19:1
Regions	5	1,489.40	10.73	2.42	3.45	>99:1
Error	45	138.84				
Total	59	Standard deviation of the experiment=11.8				

t TEST

Item	Standard error, any mean	Standard error of the difference between any 2 means	Least difference necessary for odds of—	
			19:1	99:1
Plants	4.816	6.811	13.72	18.32
Regions	3.732	5.278	10.63	14.20

The analysis of variance of the fiber diameter by plants and regions in Mexican 128 is given in table 7. The values show that there are highly significant differences between plants and also between regions.

TABLE 7.—*The analysis of variance of the fiber diameter by plants and regions on the seed coat of the Mexican 128 variety of cotton*

OBSERVED VALUES

Plant	Average fiber diameter for region No.—						Plant totals	Plant means
	1	2	3	4	5	6		
	Microns	*Microns*	*Microns*	*Microns*	*Microns*	*Microns*	*Microns*	*Microns*
A	16.49	17.80	17.54	16.75	17.54	17.54	103.7	17.28
B	15.45	15.96	15.71	14.13	14.40	14.40	90.1	15.02
C	16.23	15.96	16.49	14.92	14.66	14.92	93.2	15.53
D	18.33	17.28	16.49	16.49	17.28	17.80	103.7	17.28
E	16.49	18.33	17.54	17.02	17.28	18.06	104.7	17.45
F	16.49	17.54	17.05	15.71	15.45	14.66	96.9	16.15
G	15.96	15.71	16.23	16.49	15.18	16.49	96.1	16.02
H	16.75	16.23	14.66	15.96	13.35	16.75	93.7	15.62
I	14.40	18.33	17.02	14.66	15.71	17.02	97.1	16.18
J	16.49	17.02	16.75	17.54	15.71	16.49	100.0	16.67
Region total	163.1	170.2	165.5	159.7	156.6	164.1	979.2	
Region means	16.31	17.02	16.55	15.97	15.66	16.41		

ANALYSIS OF VARIANCE

Variation due to—	Degrees of freedom	Mean square	*F*			Odds
			Found	Required		
				5 percent point	1 percent point	
Plants	9	4.14	7.11	2.15	2.94	>99:1
Regions	5	2.22	3.81	2.42	3.45	>99:1
Error	45	.582				
Total	59	Standard deviation of the experiment=0.762				

t TEST

Item	Standard error, any mean	Standard error of the difference between any 2 means	Least difference necessary for odds of—	
			19:1	99:1
Plants	0.3110	0.4398	0.89	1.18
Regions	.2410	.3408	.69	.92

The data for the Mexican 128 variety have been presented in detailed form in tables 2, 3, 4, 5, 6, and 7 in order to give an example of the procedure used in recording the measurements of fiber population, length, weight, breaking load, percentage of thin-walled fibers, and diameter. For other varieties and for totals of all varieties, only the means and the values for the analysis of variance are given.

The values for the analysis of variance in the fiber population of the Coker-Cleveland 884–4 variety are given in table 8, wherein odds of less than 19:1 indicate no significant differences between plant means whereas odds greater than 99:1 show that differences between region means are highly significant.

TABLE 8.—*The analysis of variance of the fiber population by plants and regions on the seed coat in the Coker-Cleveland 884–4 variety of cotton*

REGION MEANS

Region No.	1	2	3	4	5	6
Mean number of fibers	2,044	437	513	273	334	241

PLANT MEANS

Plant symbol	A	B	C	D	E	F	G	H	I	J
Mean number of fibers	783	649	695	871	380	692	561	599	608	563

ANALYSIS OF VARIANCE

Variation due to—	Degrees of freedom	Mean square	*F*			Odds
			Found	Required		
				5 percent point	1 percent point	
Plants	9	108,054	1.30	2.15	2.94	<19:1
Regions	5	4,830,758	57.95	2.42	3.45	>99:1
Error	45	83,366				
Total	59	Standard deviation of the experiment = 289				

t TEST

Item	Standard error, any mean	Standard error of the difference between any 2 means	Least difference necessary for odds of—	
			19:1	99:1
Plants	118	167	336	449
Regions	91	129	[1] 260	[1] 347

[1] Probably not valid for certain comparisons. Least differences calculated from individual standard errors of regions are as follows for these comparisons:

Regions compared	Least difference for odds of—	
	19:1	99:1
2 and 4	121	166
2 and 6	106	145
3 and 4	143	196
3 and 5	145	198
3 and 6	132	180
5 and 6	81	111

The values for the fiber length in the Coker-Cleveland 884–4 variety are given in table 9, wherein odds greater than 19:1 indicate significant differences between plant means as well as region means.

TABLE 9.—*The analysis of variance of the fiber length by plants and regions on the seed coat of the Coker-Cleveland 884–4 variety of cotton*

REGION MEANS

Region No.	1	2	3	4	5	6
Mean fiber length (inches)	1.112	1.148	1.119	1.127	1.073	1.034

PLANT MEANS

Plant symbol	A	B	C	D	E	F	G	H	I	J
Mean fiber length (inches)	1.023	1.110	1.137	1.185	1.095	1.047	1.143	1.142	1.115	1.025

ANALYSIS OF VARIANCE

Variation due to—	Degrees of freedom	Mean square	*F*			Odds
			Found	Required		
				5 percent point	1 percent point	
Plants	9	0.0179	2.54	2.15	2.94	>19:1
Regions	5	.0172	2.46	2.42	3.45	>19:1
Error	45	.0070				
Total	59	Standard deviation of the experiment=0.0840				

t TEST

Item	Standard error, any mean	Standard error of the difference between any 2 means	Least difference necessary for odds of—	
			19:1	99:1
Plants	0.0343	0.0485	0.098	0.1305
Regions	.0266	.0376	.076	.1011

The variance in the fiber weight of the Coker-Cleveland 884–4 variety is shown in table 10, wherein odds of greater than 99:1 indicate that between plants and also between regions the differences are highly significant.

TABLE 10.—*The analysis of variance of the fiber weight per inch by plants and regions on the seed coat of the Coker-Cleveland 884–4 variety of cotton*

REGION MEANS

Region No.	1	2	3	4	5	6
Mean fiber weight (0.0001 mg.)	30	48	41	53	52	59

PLANT MEANS

Plant symbol	A	B	C	D	E	F	G	H	I	J
Mean fiber weight (0.0001 mg.)	52	40	44	38	56	45	41	47	57	52

TABLE 10.—*The analysis of variance of the fiber weight per inch by plants and regions on the seed coat of the Coker-Cleveland 884–4 variety of cotton*—Continued

ANALYSIS OF VARIANCE

Variation due to—	Degrees of freedom	Mean square	*F*			Odds
			Found	Required		
				5 percent point	1 percent point	
Plants	9	264.67	6.91	2.15	**2.94**	>99:1
Regions	5	1,046.20	27.31	2.42	**3.45**	>99:1
Error	45	38.31				
Total	59	Standard deviation of the experiment=6.19				

t TEST

Item	Standard error, any mean	Standard error of the difference between any 2 means	Least difference necessary for odds of—	
			19:1	99:1
Plants	2.53	3.58	7.21	9.63
Regions	1.96	2.77	5.58	7.45

The variance analysis for the fiber breaking load of the Coker-Cleveland 884–4 variety is presented in table 11, in which the odds are less than 19:1 between plants and more than 99:1 between regions.

TABLE 11.—*The analysis of variance of the fiber breaking load by plants and regions on the seed coat in the Coker-Cleveland 884–4 variety of cotton*

REGION MEANS

Region No.	1	2	3	4	5	6
Mean breaking load (grams)	2.81	4.47	3.86	5.72	5.21	5.16

PLANT MEANS

Plant symbol	A	B	C	D	E	F	G	H	I	J
Mean breaking load (grams)	4.69	4.37	4.61	4.59	4.91	4.09	4.12	4.36	5.13	4.55

ANALYSIS OF VARIANCE

Variation due to—	Degrees of freedom	Mean square	*F*			Odds
			Found	Required		
				5 percent point	1 percent point	
Plants	9	0.6357	1.57	2.15	2.94	<19:1
Regions	5	11.3737	28.15	2.42	3.45	>99:1
Error	45	.4040				
Total	59	Standard deviation of the experiment=0.636				

TABLE 11.—*The analysis of variance of the fiber breaking load by plants and regions on the seed coat in the Coker-Cleveland 884–4 variety of cotton*—Continued

t TEST

Item	Standard error, any mean	Standard error of the difference between any 2 means	Least difference necessary for odds of—	
			19:1	99:1
Plants	0.2596	0.3671	0.740	0.988
Regions	.2011	.2844	.573	.765

The data for the analysis of variance of the percentage of thin-walled fibers in the Coker-Cleveland 884–4 variety shown in table 12 indicate that there are highly significant differences between plant means and also between region means.

TABLE 12.—*The analysis of variance of the percentage of thin-walled fibers by plants and regions on the seed coat of the Coker-Cleveland 884–4 variety of cotton*

REGION MEANS

Region No.	1	2	3	4	5	6
Mean percentage of thin-walled fibers	34	14	26	11	12	10

PLANT MEANS

Plant symbol	A	B	C	D	E	F	G	H	I	J
Mean percentage of thin-walled fibers	19	27	17	17	15	12	24	23	12	13

ANALYSIS OF VARIANCE

Variation due to—	Degrees of freedom	Mean square	*F*			Odds
			Found	Required		
				5 percent point	1 percent point	
Plants	9	158.67	3.28	2.15	2.94	>99:1
Regions	5	981.80	20.28	2.42	3.45	>99:1
Error	45	48.42				
Total	59	Standard deviation of the experiment=6.96				

t TEST

Item	Standard error, any mean	Standard error of the difference between any 2 means	Least difference necessary for odds of—	
			19:1	99:1
Plants	2.841	4.018	8.09	10.81
Regions	2.201	3.113	6.27	8.37

The values for variance in the fiber diameter of the Coker-Cleveland 884–4 variety presented in table 13 do not indicate significant differences between plants or regions.

TABLE 13.—*The analysis of variance of the fiber diameter by plants and regions on the seed coat of the Coker-Cleveland 884–4 variety of cotton*

REGION MEANS

Region No.	1	2	3	4	5	6
Mean fiber diameter (microns)	15.66	15.18	15.89	15.81	15.68	15.50

PLANT MEANS

Plant symbol	A	B	C	D	E	F	G	H	I	J
Mean fiber diameter (microns)	15.36	16.01	15.14	15.31	16.23	15.58	15.36	14.83	16.41	15.97

ANALYSIS OF VARIANCE

Variation due to—	Degrees of freedom	Mean square	*F*			Odds
			Found	Required		
				5 percent point	1 percent point	
Plants	9	1.567	2.08	2.15	2.94	<19:1
Regions	5	.640	.85	4.36	9.02	<19:1
Error	45	.753				
Total	59	Standard deviation of the experiment=0.87				

t TEST

Item	Standard error, any mean	Standard error of the difference between any 2 means	Least difference necessary for odds of—	
			19:1	99:1
Plants	0.355	0.502	1.01	1.35
Regions	.275	.389	.78	1.05

The analysis of variance for the fiber population in the Farm Relief No. 1 variety is given in table 14. The odds of less than 19:1 in this table indicate no significant differences between plants, while odds greater than 99:1 point to highly significant differences between regions.

TABLE 14.—*The analysis of variance of the fiber population by plants and regions on the seed coat of the Farm Relief No. 1 variety of cotton*

REGION MEANS

Region No.	1	2	3	4	5	6
Mean number of fibers	1,493	440	361	233	261	256

TABLE 14.—*The analysis of variance of the fiber population by plants and regions on the seed coat of the Farm Relief No. 1 variety of cotton*—Continued

PLANT MEANS

Plant symbol	A	B	C	D	E	F	G	H	I	J
Mean number of fibers	493	551	497	452	439	506	550	476	593	517

ANALYSIS OF VARIANCE

Variation due to—	Degrees of freedom	Mean square	*F*			Odds
			Found	Required		
				5 percent point	1 percent point	
Plants	9	13,389	0.84	2.71	4.31	<19:1
Regions	5	2,392,653	149.58	2.42	3.45	>99:1
Error	45	15,996				
Total	59	Standard deviation of the experiment=126				

t TEST

Item	Standard error, any mean	Standard error of the difference between any 2 means	Least difference necessary for odds of—	
			19:1	99:1
Plants	51	73	147	196
Regions	40	57	[1] 115	[1] 153

[1] Probably not valid for certain comparisons. Least differences calculated from individual standard errors of regions are as follows for these comparisons:

Regions compared	Least differences for odds of—	
	19:1	99:1
3 and 4	80	109
3 and 5	86	118
3 and 6	85	117

The variance of the fiber length in the Farm Relief No. 1 variety is presented in table 15, wherein odds of greater than 99:1 show that there are highly significant differences between plants and odds greater than 19:1 indicate that there are probably significant differences in the mean fiber length of regions.

TABLE 15.—*The analysis of variance of the fiber length by plants and regions on the seed coat of the Farm Relief No. 1 variety of cotton*

REGION MEANS

Region No.	1	2	3	4	5	6
Mean fiber length (inches)	1.130	1.181	1.190	1.197	1.153	1.140

TABLE 15.—*The analysis of variance of the fiber length by plants and regions on the seed coat of the Farm Relief No. 1 variety of cotton*—Continued

PLANT MEANS

Plant symbol	A	B	C	D	E	F	G	H	I	J
Mean fiber length (inches)	1.170	1.180	1.120	1.160	1.120	1.050	1.290	1.230	1.140	1.200

ANALYSIS OF VARIANCE

Variation due to—	Degrees of freedom	Mean square	*F*			Odds
			Found	Required		
				5 percent point	1 percent point	
Plants	9	0.0265	8.55	2.15	2.94	>99:1
Regions	5	.0078	2.52	2.42	3.45	>19:1
Error	45	.0031				
Total	59	Standard deviation of the experiment=0.0557				

t TEST

Item	Standard error, any mean	Standard error of the difference between any 2 means	Least difference necessary for odds of—	
			19:1	99:1
Plants	0.0227	0.0321	0.0646	0.0863
Regions	.0176	.0249	.0501	.0670

The analysis of variance of the fiber weight in the Farm Relief No. 1 variety is given in table 16. The odds of greater than 99:1 therein shown indicate highly significant differences between plants and also between regions.

TABLE 16.—*The analysis of variance of the fiber weight per inch by plants and regions on the seed coat of the Farm Relief No. 1 variety of cotton*

REGION MEANS

Region No.	1	2	3	4	5	6
Mean fiber weight (0.0001 mg.)	34	52	48	59	56	57

PLANT MEANS

Plant symbol	A	B	C	D	E	F	G	H	I	J
Mean fiber weight (0.0001 mg.)	54	40	49	61	52	65	44	47	45	53

TABLE 16.—*The analysis of variance of the fiber weight per inch by plants and regions on the seed coat of the Farm Relief No. 1 variety of cotton*—Continued

ANALYSIS OF VARIANCE

Variation due to—	Degrees of freedom	Mean square	*F*			Odds
			Found	Required		
				5 percent point	1 percent point	
Plants	9	366.33	8.77	2.15	2.94	>99:1
Regions	5	866.60	20.75	2.42	3.45	>99:1
Error	45	41.76				
Total	59	Standard deviation of the experiment=6.46				

t TEST

Item	Standard error, any mean	Standard error of the difference between any 2 means	Least difference necessary for odds of—	
			19:1	99:1
Plants	2.64	3.73	7.51	10.03
Regions	2.04	2.88	5.80	7.75

The data for variance of fiber breaking load in the Farm Relief No. 1 variety presented in table 17 indicate nonsignificant differences between plants and highly significant differences between regions.

TABLE 17.—*The analysis of variance of the fiber breaking load by plants and regions on the seed coat of the Farm Relief No. 1 variety of cotton*

REGION MEANS

Region No.	1	2	3	4	5	6
Mean breaking load (grams)	3.04	4.52	4.20	5.78	5.45	5.49

PLANT MEANS

Plant symbol	A	B	C	D	E	F	G	H	I	J
Mean breaking load (grams)	4.35	4.81	4.96	4.25	4.64	4.90	4.74	4.58	5.50	4.73

ANALYSIS OF VARIANCE

Variation due to—	Degrees of freedom	Mean square	*F*			Odds
			Found	Required		
				5 percent point	1 percent point	
Plants	9	0.7260	1.40	2.15	2.94	<19:1
Regions	5	10.7310	20.63	2.42	3.45	>99:1
Error	45	.5202				
Total	59	Standard deviation of the experiment=0.721				

TABLE 17.—*The analysis of variance of the fiber breaking load by plants and regions on the seed coat of the Farm Relief No. 1 variety of cotton*—Continued

t TEST

Item	Standard error, any mean	Standard error of the difference between any 2 means	Least difference necessary for odds of—	
			19:1	99:1
Plants	0.294	0.416	0.838	1.119
Regions	.228	.322	.649	.866

Values for the analysis of variance in the percentage of thin-walled fibers in the Farm Relief No. 1 variety are presented in table 18. Odds greater than 99:1 point to the fact that there are highly significant differences between plant and also region means in this character.

TABLE 18.—*The analysis of variance of the percentage of thin-walled fibers by plants and regions on the seed coat of the Farm Relief No. 1 variety of cotton*

REGION MEANS

Region No.	1	2	3	4	5	6
Mean percentage of thin-walled fibers	59	11	24	6	7	12

PLANT MEANS

Plant symbol	A	B	C	D	E	F	G	H	I	J
Mean percentage of thin-walled fibers	13	32	7	14	13	13	36	29	30	12

ANALYSIS OF VARIANCE

Variation due to—	Degrees of freedom	Mean square	Found	Required		Odds
				5 percent point	1 percent point	
Plants	9	677.11	4.48	2.15	2.94	>99:1
Regions	5	4,166.20	27.59	2.42	3.45	>99:1
Error	45	151.02				
Total	59	Standard deviation of the experiment = 12.29				

t TEST

Item	Standard error any mean	Standard error of the differences between any 2 means	Least difference necessary for odds of—	
			19:1	99:1
Plants	5.02	7.10	14.30	19.10
Regions	3.89	5.50	11.08	14.80

The variance analysis for fiber diameter of the Farm Relief No. 1 variety in table 19 indicates highly significant differences between plants and nonsignificant differences between regions.

TABLE 19.—*The analysis of variance of the fiber diameter by plants and regions on the seed coat in the Farm Relief No. 1 variety of cotton*

REGION MEANS

Region No	1	2	3	4	5	6
Mean fiber diameter (microns)	17.25	17.04	17.04	16.34	16.70	16.75

PLANT MEANS

Plant symbol	A	B	C	D	E	F	G	H	I	J
Mean fiber diameter (microns)	17.19	16.80	15.84	16.76	16.54	17.89	16.93	17.72	16.75	16.14

ANALYSIS OF VARIANCE

Variation due to—	Degrees of freedom	Mean square	*F*			Odds
			Found	Required		
				5 percent point	1 percent point	
Plants	9	2.389	4.68	2.15	2.94	>99:1
Regions	5	1.090	2.08	2.42	3.45	<19:1
Error	45	.51				
Total	59	Standard deviation of the experiment=0.714				

t TEST

Item	Standard error, any mean	Standard error of the difference between any 2 means	Least difference necessary for odds of—	
			19:1	99:1
Plants	0.291	0.412	0.83	1.11
Regions	.226	.320	.64	.86

The variance analysis for the distribution of the fiber population on the seed coat of the Acala 4067 variety is presented in table 20, wherein odds less than 19:1 indicate that differences between plants are not significant and odds greater than 99:1 show that there are highly significant differences between regions.

TABLE 20.—*The analysis of variance of the fiber population by plants and regions on the seed of the Acala 4067 variety of cotton*

REGION MEANS

Region No	1	2	3	4	5	6
Mean number of fibers	1,646	499	753	270	395	406

TABLE 20.—*The analysis of variance of the fiber population by plants and regions on the seed of the Acatla 4067 variety of cotton*—Continued

PLANT MEANS

Plant symbol	A	B	C	D	E	F	G	H	I	J
Mean number of fibers	724	585	433	569	1,035	790	567	701	504	707

ANALYSIS OF VARIANCE

Variation due to—	Degrees of freedom	Mean square	*F*			Odds
			Found	Required		
				5 percent point	1 percent point	
Plants	9	175,836	1.91	2.15	2.94	<19:1
Regions	5	2,586,997	28.06	2.42	3.45	>99:1
Error	45	92,187				
Total	59	Standard deviation of the experiment=304				

t TEST

Item	Standard error, any mean	Standard error of the difference between any 2 means	Least difference necessary for odds of—	
			19:1	99:1
Plants	124	175	352	471
Regions	96	136	[1] 274	[1] 366

[1] Probably not valid for certain comparisons. Least differences calculated from individual standard errors of regions are as follows for these comparisons:

Regions compared	Least difference for odds of—	
	19:1	99:1
2 and 4	122	167
3 and 4	387	530
3 and 5	387	530
3 and 6	384	527
4 and 5	100	136
4 and 6	86	118

The variance analysis for the distribution of the fiber length on the seed coat of the Acala 4067 variety is presented in table 21, wherein odds of greater than 99:1 indicate that there are highly significant differences between region means and also between plant means.

TABLE 21.—*The analysis of variance of the fiber length by plants and regions on the seed coat of the Acala 4067 variety of cotton*

REGION MEANS

Region No.	1	2	3	4	5	6
Mean fiber length (inches)	1.030	1.016	1.001	0.982	0.943	0.942

PLANT MEANS

Plant symbol	A	B	C	D	E	F	G	H	I	J
Mean fiber length (inches)	1.000	1.050	1.050	0.960	0.990	1.000	0.960	0.830	0.960	1.060

ANALYSIS OF VARIANCE

Variance due to—	Degrees of freedom	Mean square	*F*			Odds
			Found	Required		
				5 percent point	1 percent point	
Plants	9	0.0268	9.93	2.15	2.94	>99:1
Regions	5	.0137	5.07	2.42	3.45	>99:1
Error	45	.0027				
Total	59	Standard deviation of the experiment=0.052				

t TEST

Item	Standard error, any mean	Standard error of the difference between any 2 means	Least difference necessary for odds of—	
			19:1	99:1
Plants	0.0212	0.0300	0.0604	0.0807
Regions	.0164	.0232	.0467	.0624

The analysis of variance for the fiber weight in the Acala 4067 variety is presented in table 22. Odds greater than 99:1 point to highly significant differences between both regions and plants.

TABLE 22.—*The analysis of variance for the fiber weight per inch by plant and regions on the seed coat of the Acala 4067 variety of cotton*

REGION MEANS

Region No.	1	2	3	4	5	6
Mean fiber weight (0.0001 mg.)	33	43	41	58	55	58

PLANT MEANS

Plant symbol	A	B	C	D	E	F	G	H	I	J
Mean fiber weight (0.0001 mg.)	40	48	48	49	42	46	47	51	65	46

TABLE 22.—*The analysis of variance for the fiber weight per inch by plant and regions on the seed coat of the Acala 4067 variety of cotton*—Continued

ANALYSIS OF VARIANCE

Variance due to—	Degrees of freedom	Mean square	*F*			Odds
			Found	Required		
				5 percent point	1 percent point	
Plants	9	260.33	8.72	2.15	2.94	>99:1
Regions	5	1,107.40	37.07	2.42	3.45	>99:1
Error	45	29.87				
Total	59	Standard deviation of the experiment=5.465				

t TEST

Item	Standard error, any mean	Standard error of the difference between any 2 means	Least difference necessary for odds of—	
			19:1	99:1
Plants	2.23	3.15	6.34	8.47
Regions	1.73	2.45	4.93	6.59

The analysis of variance for the fiber breaking load in the Acala 4067 variety is presented in table 23. The values show that there are highly significant differences between plants and also between regions in the fiber strength.

TABLE 23.—*The analysis of variance of the fiber breaking load by plants and regions on the seed coat of the Acala 4067 variety of cotton*

REGION MEANS

Region No.	1	2	3	4	5	6
Mean breaking load (grams)	3.16	4.23	4.22	6.28	5.59	6.01

PLANT MEANS

Plant symbol	A	B	C	D	E	F	G	H	I	J
Mean breaking load (grams)	3.79	4.60	4.37	5.22	4.16	5.91	5.61	4.75	5.46	5.29

ANALYSIS OF VARIANCE

Variation due to—	Degrees of freedom	Mean square	*F*			Odds
			Found	Required		
				5 percent point	1 percent point	
Plants	9	2.8487	4.53	2.15	2.94	>99:1
Regions	5	15.0388	23.90	2.42	3.45	>99:1
Error	45	.6293				
Total	59	Standard deviation of the experiment=0.793				

TABLE 23.—*The analysis of variance of the fiber breaking load by plants and regions on the seed coat of the Acala 4067 variety of cotton*—Continued

t TEST

Item	Standard error, any mean	Standard error of the difference between any 2 means	Least difference necessary for odds of—	
			19:1	99:1
Plants	0.3237	0.4578	0.922	1.231
Regions	.2508	.3547	.714	.954

The variance analysis of the percentage of thin-walled fibers in the Acala 4067 variety is contained in table 24 wherein the values indicate real differences between regions and nonsignificant differences between plants for the percentage means.

TABLE 24.—*The analysis of variance of the percentage of thin-walled fibers by plants and regions on the seed coat of the Acala 4067 variety of cotton*

REGION MEANS

Region No.	1	2	3	4	5	6
Mean percentage of thin-walled fibers	59	24	34	4	9	6

PLANT MEANS

Plant symbol	A	B	C	D	E	F	G	H	I	J
Mean percentage of thin-walled fibers	30	18	34	11	32	25	16	23	19	18

ANALYSIS OF VARIANCE

Variation due to—	Degrees of freedom	Mean square	*F*			Odds
			Found	Required		
				5 percent point	1 percent point	
Plants	9	344.00	1.91	2.15	2.94	<19:1
Regions	4	4,510.40	25.07	2.42	3.45	>99:1
Error	45	179.89				
Total	59	Standard deviation of the experiment=13.40				

t TEST

Item	Standard error, any mean	Standard error of the difference between any 2 means	Least difference necessary for odds of—	
			19:1	99:1
Plants	5.47	7.74	15.59	20.82
Regions	4.24	5.99	12.06	16.11

The variance values for the Acala 4067 variety presented in table 25 indicate highly significant differences in fiber diameter between plants and significant differences between regions.

TABLE 25.—*The analysis of variance of the fiber diameter by plants and regions on the seed coat of the Acala 4067 variety of cotton*

REGION MEANS

Region No.	1	2	3	4	5	6
Mean fiber diameter (microns)	15.81	15.16	14.55	15.10	15.10	14.82

PLANT MEANS

Plant symbol	A	B	C	D	E	F	G	H	I	J
Mean fiber diameter (microns)	14.49	15.23	14.96	15.97	15.27	14.79	14.44	14.26	16.62	14.88

ANALYSIS OF VARIANCE

Variation due to—	Degrees of freedom	Mean square	*F*			Odds
			Found	Required		
				5 percent point	1 percent point	
Plants	9	3.200	6.15	2.15	2.94	>99:1
Regions	5	1.760	3.38	2.42	3.45	>99:1
Error	45	.520				
Total	59	Standard deviation of the experiment=0.721				

t TEST

Item	Standard error, any mean	Standard error of the difference between any 2 means	Least difference necessary for odds of—	
			19:1	99:1
Plants	0.294	0.416	0.84	1.12
Regions	.228	.322	.65	.87

The variance of the fiber population in the Rowden 40 variety is contained in Table 26. Odds of less than 19:1 indicate that differences between plants are not significant, whereas odds greater than 99:1 show that there are highly significant differences between regions.

TABLE 26.—*The analysis of variance of the fiber population by plants and regions on the seed coat of the Rowden 40 variety of cotton*

REGION MEANS

Region No.	1	2	3	4	5	6
Mean number of fibers	1,725	414	424	202	438	216

TABLE 26.—*The analysis of variance of the fiber population by plants and regions on the seed coat of the Rowden 40 variety of cotton*—Continued

PLANT MEANS

Plant symbol	A	B	C	D	E	F	G	H	I	J
Mean number of fibers	466	564	627	579	472	719	440	599	750	481

ANALYSIS OF VARIANCE

Variation due to—	Degrees of freedom	Mean square	*F*			Odds
			Found	Required		
				5 percent point	1 percent point	
Plants	9	69,446	.64	2.71	4.31	<19:1
Regions	5	3,315,923	30.59	2.42	3.45	>99:1
Error	45	108,415				
Total	59	Standard deviation of the experiment=329				

t TEST

Item	Standard error, any mean	Standard error of the difference between any 2 means	Least difference necessary for odds of—	
			19:1	99:1
Plants	134	190	383	511
Regions	104	147	[1] 296	[1] 395

[1] Probably not valid for certain comparisons. Least differences calculated from individual standard errors of regions are as follows for these comparisons:

Regions compared	Least differences for odds of—	
	19:1	99:1
2 and 4	116	158
2 and 6	127	174
3 and 4	124	169
3 and 6	134	184

The analysis of variance for the fiber length in the Rowden 40 variety is presented in table 27, wherein odds greater than 99:1 indicate that there are highly significant differences between both regions and plants.

TABLE 27.—*The analysis of variance of the fiber length by plants and regions on the seed coat of the Rowden 40 variety of cotton*

REGION MEANS

Region No.	1	2	3	4	5	6
Mean fiber length (inches)	1.053	1.015	1.000	0.992	0.947	0.937

TABLE 27.—*The analysis of variance of the fiber length by plants and regions on the seed coat of the Rowden 40 variety of cotton*—Continued

PLANT MEANS

Plant symbol	A	B	C	D	E	F	G	H	I	J
Mean fiber length (inches)	1.090	0.950	1.060	1.010	1.020	0.860	0.910	1.060	0.890	1.060

ANALYSIS OF VARIANCE

Variation due to—	Degrees of freedom	Mean square	*F*			Odds
			Found	Required		
				5 percent point	1 percent point	
Plants	9	0.0421	14.52	2.15	2.94	>99:1
Regions	5	.0187	6.45	2.42	3.45	>99:1
Error	45	.0029				
Total	59	Standard deviation of the experiment =0.054.				

t TEST

Item	Standard error, any mean	Standard error of the difference between any 2 means	Least difference necessary for odds of—	
			19:1	99:1
Plants	0.0220	0.0311	0.0626	0.0837
Regions	.0171	.0242	.0487	.0651

The analysis of variance for the fiber weight in the Rowden 40 variety is contained in table 28. Odds greater than 19:1 for plants and more than 99:1 for regions show that there are significant differences between both regions and plants.

TABLE 28.—*The analysis of variance of the fiber weight per inch by plants and regions on the seed coat of the Rowden 40 variety of cotton*

REGION MEANS

Region No.	1	2	3	4	5	6
Mean fiber weight (0.0001 mg.)	38	48	45	65	57	65

PLANT MEANS

Plant symbol	A	B	C	D	E	F	G	H	I	J
Mean fiber weight (0.0001 mg.)	35	41	57	51	56	53	55	73	60	48

TABLE 28.—*The analysis of variance of the fiber weight per inch by plants and regions on the seed coat of the Rowden 40 variety of cotton*—Continued

ANALYSIS OF VARIANCE

Variation due to—	Degrees of freedom	Mean square	*F*			Odds
			Found	Required		
				5 percent point	1 percent point	
Plants	9	639.78	2.80	2.15	2.94	>19:1
Regions	5	1,293.60	5.66	2.42	3.45	>99:1
Error	45	228.56				
Total	59	Standard deviation of the experiment=15.12.				

t TEST

Item	Standard error, any mean	Standard error of the difference between any 2 means	Least difference necessary for odds of—	
			19:1	99:1
Plants	6.17	8.73	17.58	23.48
Regions	4.78	6.76	13.61	18.18

The variance analysis for fiber breaking load in the Rowden 40 variety presented in table 29 indicates very real differences between plants and also between regions.

TABLE 29.—*The analysis of variance of the fiber breaking load by plants and regions on the seed coat of the Rowden 40 variety of cotton*

REGION MEANS

Region No	1	2	3	4	5	6
Mean breaking load (grams)	4.30	4.90	4.54	6.72	6.11	6.12

PLANT MEANS

Plant symbol	A	B	C	D	E	F	G	H	I	J
Mean breaking load (grams)	6.46	4.43	4.38	4.25	7.32	4.28	6.21	4.84	6.25	6.04

ANALYSIS OF VARIANCE

Variation due to—	Degrees of freedom	Mean square	*F*			Odds
			Found	Required		
				5 percent point	1 percent point	
Plants	9	7.6398	8.46	2.15	2.94	>99:1
Regions	5	9.9125	10.98	2.42	3.45	>99:1
Error	45	.9028				
Total	59	Standard deviation of the experiment=0.95.				

TABLE 29.—*The analysis of variance of the fiber breaking load by plants and regions on the seed coat of the Rowden 40 variety of cotton*—Continued

t TEST

Item	Standard error, any mean	Standard error of the difference between any 2 means	Least difference necessary for odds of—	
			19:1	99:1
Plants	0.3878	0.5484	1.104	1.475
Regions	.3004	.4248	.856	1.143

The data contained in table 30 for the variance of the percentage of thin-walled fibers in the Rowden 40 variety indicate that there are very real differences between regions and nonsignificant differences between plants.

TABLE 30.—*The analysis of variance of the percentage of thin-walled fibers by plants and regions on the seed coat of the Rowden 40 variety of cotton*

REGION MEANS

Region No.	1	2	3	4	5	6
Mean percentage of thin-walled fibers	62	30	32	9	9	9

PLANT MEANS

Plant symbol	A	B	C	D	E	F	G	H	I	J
Mean percentage of thin-walled fibers	14	21	24	27	25	38	22	36	20	25

ANALYSIS OF VARIANCE

Variation due to—	Degrees of freedom	Mean square	*F*			Odds
			Found	Required		
				5 percent point	1 percent point	
Plants	9	310.33	1.15	2.15	2.94	<19:1
Regions	5	4,441.20	16.52	2.42	3.45	>99:1
Error	45	268.91				
Total	59	Standard deviation of the experiment=16.40.				

t TEST

Item	Standard error, any mean	Standard error of the difference between any 2 means	Least difference necessary for odds of—	
			19:1	99:1
Plants	6.69	9.46	19.05	25.45
Regions	5.19	7.34	14.78	19.74

The variance analysis for fiber diameter in the Rowden 40 variety is presented in table 31. The values do not point to significant differences between either plants or regions.

VARIANCE OF FIBER CHARACTERS FOR THE ENTIRE EXPERIMENT

Results for the analysis of variance within varieties have been given on the preceding pages.

The whole experiment has been set up and analyzed for variance of fiber population, length, weight, breaking load, percentage of thin-walled fibers, and diameter.

TABLE 31.—*The analysis of variance of the fiber diameter by plants and regions on the seed coat in the Rowden 40 variety of cotton*

REGION MEANS

Region No.	1	2	3	4	5	6
Mean fiber diameter (microns)	16.96	16.52	16.55	16.28	16.78	16.52

PLANT MEANS

Plant symbol	A	B	C	D	E	F	G	H	I	J
Mean fiber diameter (microns)	16.53	16.62	16.79	16.32	17.06	16.58	16.10	17.10	16.45	16.45

ANALYSIS OF VARIANCE

Variation due to—	Degrees of freedom	Mean square	*F*			Odds
			Found	Required		
				5 percent point	1 percent point	
Plants	9	0.589	1.26	2.15	2.94	<19:1
Regions	5	.560	1.19	2.42	3.45	<19:1
Error	45	.469				
Total	59	Standard deviation of the experiment=0.685				

t TEST

Item	Standard error, any mean	Standard error of the difference between any 2 means	Least difference necessary for odds of—	
			19:1	99:1
Plants	0.280	0.396	0.798	1.065
Regions	.217	.307	.618	.826

The analysis of variance for the fiber population of the whole experiment is given in table 32, wherein odds of more than 19:1 indicate that there are significant differences between the mean populations of varieties and odds greater than 99:1 show that there are highly significant differences between the mean populations of regions. Odds amounting to more than 19:1 point to the fact that the interac-

tion of varieties with regions (varieties times regions)[3] is significant; that is, varieties significantly affected the fiber population of regions.

TABLE 32.—*The analysis of variance of the fiber population for the total experiment including all five varieties of cotton*

REGION MEANS

Region No.	1	2	3	4	5	6
Mean number of fibers	1,673	417	505	253	351	287

VARIETY MEANS

	Mexican 128	Coker-Cleveland 884–4	Farm Relief No. 1	Acala 4067	Rowden 40
Mean number of fibers per region	529	640	507	662	570

ANALYSIS OF VARIANCE

Source of variation	Degrees of freedom	Mean square	*F*			Odds
			Found	Required		
				5 percent point	1 percent point	
Between varieties	4	277,205	[1] 3.38	2.58	3.77	>19:1
Within varieties	45	82,074				
Between regions	5	14,718,628	[2] 110.16	2.71	4.10	>99:1
Varieties×regions	20	133,607	[3] 1.73	1.57	1.88	>19:1
Remainder	225	77,248				
Total	299					

t TEST

Item	Standard error, any mean	Standard error of the difference between any 2 means	Least difference necessary for odds of—	
			19:1	99:1
Varieties [4]	37	52	105	140
Regions	52	74	[5] 154	[5] 211

[1] $\frac{\text{M. S. varieties}}{\text{M. S. within varieties}}$.

[2] $\frac{\text{M. S. regions}}{\text{M. S. varieties×regions}}$.

[3] $\frac{\text{M. S. varieties×regions}}{\text{M. S. remainder}}$.

[4] n for computing standard error any mean=60; n would be 10 if means were plant totals.

[5] Probably not valid for certain comparisons. Least differences calculated from individual standard errors of regions are as follows for these comparisons:

Regions computed	Least differences for odds of—	
	19:1	99:1
2 and 4	55	73
2 and 6	54	71
3 and 5	121	161
4 and 5	83	110

The variance analysis of the fiber length for the whole experiment is contained in table 33, wherein odds greater than 99:1 point to the fact that between varieties and also between regions on the seed coat there are highly significant differences. In table 33, also, odds of less than 19:1 indicate that the interaction of varieties and regions is not significant for length; that is, varieties apparently did not significantly affect the fiber length of the regions on the seed.

TABLE 33.—*The analysis of variance of the fiber length of the total experiment including all five varieties of cotton*

REGION MEANS

Region No.	1	2	3	4	5	6
Mean fiber length (inches)	1.094	1.097	1.085	1.079	1.044	1.029

VARIETY MEANS

	Mexican 128	Coker-Cleveland 884–4	Farm Relief No. 1	Acala 4067	Rowden 40
Mean fiber length per region (inches)	1.113	1.102	1.165	0.986	0.991

ANALYSIS OF VARIANCE

Source of variation	Degrees of freedom	Mean square	*F*			Odds
			Found	Required		
				5 percent point	1 percent point	
Between varieties	4	0.3801	[1] 11.45	2.58	3.77	>99:1
Within varieties	45	.0332				
Between regions	5	.0393	[2] 8.93	2.26	3.11	>99:1
Varieties×regions	20	.0054	[3] 1.23	1.57	1.88	<19:1
Remainder	225	.0044				
Total	299					

t TEST

Item	Standard error any mean	Standard error of the difference between any 2 means	Least difference necessary for odds of—	
			19:1	99:1
Varieties [4]	0.0235	0.0333	0.0671	0.0896
Regions	.0094	.0133	.0262	.0346

[1] $\frac{\text{M. S. varieties}}{\text{M. S. within varieties.}}$.

[2] $\frac{\text{M. S. regions}}{\text{M. S. remainder.}}$. Remainder is used as error for regions instead of varieties×regions, which is not significant relative to the remainder.

[3] $\frac{\text{M. S. varieties} \times \text{regions}}{\text{M. S. remainder}}$.

[4] *n* for computing standard error any mean=60; *n* would be 10 if means were plant totals.

The variance analysis for the fiber weight per inch of the whole experiment is presented in table 34, wherein odds less than 19:1 indicate that the differences between varieties in the fiber weight (average weight of the six regions on the seed coat) are not significant. Odds of greater than 99:1 in table 34 show that differences between regions are highly significant, while odds less than 19:1 point to the fact that varieties did not significantly influence the fiber weight per inch of regions.

TABLE 34.—*The analysis of variance of the fiber weight per inch for the whole experiment including all five varieties of cotton*

REGION MEANS

Region No.	1	2	3	4	5	6
Mean fiber weight (0.0001 mg.)	34	49	44	58	55	59

VARIETY MEANS

	Mexican 128	Coker-Cleveland 884-4	Farm Relief No. 1	Acala 4067	Rowden 40
Mean fiber weight (0.0001 mg.)	50	47	51	48	53

ANALYSIS OF VARIANCE

Source of variation	Degrees of freedom	Mean square	*F*			Odds
			Found	Required		
				5 percent point	1 percent point	
Between varieties	4	309.50	[1] 0.92	5.63	13.46	<19:1
Within varieties	45	335.38				
Between regions	5	4,523.00	[2] 56.88	2.26	3.11	>99:1
Varieties×regions	20	93.50	[3] 1.18	1.57	1.88	<19:1
Remainder	225	79.52				
Total	299					

t TEST

Item	Standard error, any mean	Standard error of the difference between any 2 means	Least difference necessary for odds of—	
			19:1	99:1
Varieties [4]	2.363	3.342	6.731	8.990
Regions	1.263	1.784	3.518	4.640

[1] $\frac{\text{M. S. varieties}}{\text{M. S. within varieties.}}$

[2] $\frac{\text{M. S. regions}}{\text{M. S. remainder.}}$ Remainder is used as error for regions instead of varieties×regions, which is not significant relative to the remainder.

[3] $\frac{\text{M. S. varieties} \times \text{regions.}}{\text{M. S. remainder}}$

[4] n for computing standard error any mean=60; n would be 10 if means were plant totals.

The variance analysis for the single fiber breaking load of the entire experiment is contained in table 35, wherein odds greater than 19:1 indicate significant differences between varieties. Odds of more than 99:1 show that there are highly significant differences between region means, while odds less than 19:1 point to the fact that varieties did not significantly influence the fiber breaking load of regions.

TABLE 35.—*The analysis of variance of the fiber breaking load for the total experiment including all five varieties of cotton*

REGION MEANS

Region No.	1	2	3	4	5	6
Mean breaking load (grams)	3.44	4.73	4.32	6.11	5.70	5.78

VARIETY MEANS

	Mexican 128	Coker-Cleveland 884-4	Farm Relief No. 1	Acala 4067	Rowden 40
Mean fiber breaking load (grams)	5.42	4.54	4.75	4.92	5.45

ANALYSIS OF VARIANCE

Source of variation	Degrees of freedom	Mean square	*F*			Odds
			Found	Required		
				5 percent point	1 percent point	
Between varieties	4	9.8257	[1] 3.56	2.58	3.77	>19:1
Within varieties	45	2.7567				
Between regions	5	53.2078	[2] 85.06	2.26	3.11	>99:1
Varieties×regions	20	.6044	[3] .97	1.84	2.42	<19:1
Remainder	225	.6255				
Total	299					

t TEST

Item	Standard error, any mean	Standard error of the difference between any 2 means	Least difference necessary for odds of—	
			19:1	99:1
Varieties [4]	0.2142	0.3029	0.6100	0.8148
Regions	.1119	.1582	.3120	.4115

[1] $\frac{\text{M. S. varieties}}{\text{M. S. within varieties}}$.

[2] $\frac{\text{M. S. Regions}}{\text{M. S. remainder}}$. Remainder is used as error for regions instead of varieties×regions, which is not significant relative to the remainder.

[3] $\frac{\text{M. S. varieties×regions.}}{\text{M. S. remainder}}$.

[4] n for computing standard error any mean=60; n would be 10 if means were plant totals.

The analysis of variance of the percentage of thin-walled fibers for the total experiment, presented in table 36, gives odds greater than 19:1 for varieties and odds greater than 99:1 for regions, and also for

varieties×regions; that is, there are differences between both varieties and regions, and varieties significantly affected regions relative to the percentage of thin-walled fibers.

TABLE 36.—*The analysis of variance of the percentage of thin-walled fibers for the total experiment including all five varieties of cotton*

REGION MEANS

Region No.	1	2	3	4	5	6
Mean percentage of thin-walled fibers	50	19	28	8	9	8

VARIETY MEANS

	Mexican 128	Coker-Cleveland 884-4	Farm Relief No. 1	Acala 4067	Rowden 40
Mean percentage of thin-walled fibers	16	18	20	23	25

ANALYSIS OF VARIANCE

Source of variation	Degrees of freedom	Mean square	*F*			Odds
			Found	Required		
				5 percent point	1 percent point	
Between varieties	4	836.96	[1] 2.61	2.58	3.77	>19:1
Within varieties	45	320.67				
Between regions	5	13,933.49	[2] 33.66	2.71	4.10	>99:1
Varieties×regions	20	413.94	[3] 2.63	1.57	1.88	>99:1
Remainder	225	157.41				
Total	299					

t TEST

Item	Standard error, any mean	Standard error of the difference between any 2 means	Least difference necessary for odds of—	
			19:1	99:1
Varieties	2.311	3.268	6.58	8.79
Regions	2.877	4.069	8.49	11.58

[1] $\frac{\text{M. S. varieties}}{\text{M. S. within varieties}}$.

[2] $\frac{\text{M. S. regions}}{\text{M. S. varieties×regions}}$.

[3] $\frac{\text{M. S. varieties×regions}}{\text{M. S. remainder}}$.

[4] n for computing standard error, any mean=60; n would be 10 if means were plant totals.

The analysis of variance of the fiber diameter for the entire experiment is given in table 37. Odds greater than 99:1 in this table indicate that there are highly significant differences between varieties and also point to the fact that varieties very significantly influenced the fiber diameter of regions. Odds of less than 19:1 in the same table indicate that differences between regions are not significant.

TABLE 37.—*The analysis of variance of the fiber diameter for the total experiment including all five varieties of cotton*

REGION MEANS

Region No.	1	2	3	4	5	6
Mean fiber diameter (microns)	16.40	16.18	16.12	15.90	15.99	16.00

VARIETY MEANS

	Mexican 128	Coker-Cleveland 884-4	Farm Relief No. 1	Acala 4067	Rowden 40
Mean fiber diameter (microns)	16.32	15.62	16.85	15.09	16.60

ANALYSIS OF VARIANCE

Source of variation	Degrees of freedom	Mean square	*F*			Odds
			Found	Required		
				5 percent point	1 percent point	
Between varieties	4	31.81	[1] 13.37	2.58	3.77	>99:1
Within varieties	45	2.38				
Between regions	5	1.59	[2] 1.36	2.71	4.10	<19:1
Varieties×regions	20	1.17	[3] 2.05	1.57	1.88	>99:1
Remainder	225	.57				
Total	299					

t TEST

Item	Standard error, any mean	Standard error of the difference between any 2 means	Least difference necessary for odds of—	
			19:1	99:1
Varieties [4]	0.1991	0.2816	0.57	0.76
Regions	.1530	.2164	.45	.62

[1] $\frac{\text{M. S. varieties}}{\text{M. S. within varieties}}$.

[2] $\frac{\text{M. S. regions}}{\text{M. S. varieties×regions}}$.

[3] $\frac{\text{M. S. varieties×regions}}{\text{M. S. remainder}}$.

[4] n for computing standard error any mean=60; n would be 10 if means were plant totals.

COMPARISON OF THE FIBER POPULATION OF VARIETIES BY SINGLE REGIONS

From data on the fiber population of each of the six regions from the 10 plants of each of the five varieties calculations were made to obtain the analysis of variance values for population. These summarized values are presented in table 38. Between varieties the odds shown are less than 19:1 for regions 1, 3, and 4, 19:1 for region 2, and greater than 99:1 for regions 5 and 6.

TABLE 38.—*The variance of fiber population by single regions for between the five varieties of cotton*

OBSERVED VALUES

Varieties	Mean fiber population for region No.—					
	1	2	3	4	5	6
Mexican 128	1,457	296	476	286	327	317
Coker-Cleveland 884-4	2,044	437	513	273	334	241
Farm Relief No. 1	1,493	440	361	233	261	256
Acala 4067	1,646	499	753	270	395	406
Rowden 40	1,725	414	421	202	438	216

ANALYSIS OF VARIANCE

Region No.—	Variation due to—	Degrees of freedom	Mean square	*F*			Odds
				Found	Required		
					5 percent point	1 percent point	
1	Varieties	4	549,268	2.31	2.58	2.77	<19:1
	Error	45	238,022				
	Total	49					
2	Varieties	4	55,702	2.582	2.58	3.77	19:1
	Error	45	21,575				
	Total	49					
3	Varieties	4	224,216	2.06	2.58	3.77	<19:1
	Error	45	109,087				
	Total	49					
4	Varieties	4	11,843	.77	5.63	13.46	<19:1
	Error	45	15,380				
	Total	49					
5	Varieties	4	682,924	34.95	2.58	3.77	>99:1
	Error	45	19,539				
	Total	49					
6	Varieties	4	57,931	7.17	2.58	3.77	>99:1
	Error	45	8,085				
	Total	49					

t TEST

Region No.—	Standard error, any mean	Standard error of the difference between any 2 means	Least difference necessary for odds of—	
			19:1	99:1
1	154	218	439	586
2	46	65	131	175
3	105	148	298	398
4	39	55	111	148
5	44	63	127	169
6	29	40	81	108

COMPARISON OF VARIETIES FOR GOODNESS OF FIT FOR FIBER DISTRIBUTION

The value of χ^2 was determined for each of 10 possible pairs of varieties from the formula [10]

$$\chi^2=\left[\sum\frac{(f_a/N_a-f_b/N_b)^2}{f_a+f_b}\right]N_aN_b$$

in which f_a and f_b are the frequencies and N_a and N_b are the totals of these frequencies. The distribution of the fiber population, χ^2, and P values are presented in table 39. The P values in this table are less than 0.000005 and the odds greater than 199,999:1 for any pair of varieties; therefore the distribution of fiber population is different in each possible comparison.

TABLE 39.—*The goodness of fit for the fiber distribution of all five cotton varieties*

OBSERVED VALUES

Region No. or class [1]	Average fiber population of variety named—				
	Mexican 128	Coker-Cleveland 884-4	Farm Relief No. 1	Acala 4067	Rowden 40
1	1,457	2,044	1,493	1,646	1,725
2	296	437	440	499	414
3	477	513	361	753	424
4	286	273	233	270	202
5	327	334	261	395	438
6	317	241	256	406	216

χ^2 VALUE

Varieties compared	Value of χ^2	Value of P	Odds
Mexican 128 and Coker-Cleveland 884-4	71.87	0.000005	Greater than 199,999:1.
Mexican 128 and Farm Relief No. 1	61.75		
Mexican 128 and Acala 4067	51.92		
Mexican 128 and Rowden 40	84.70		
Coker-Cleveland 884-4 and Farm Relief No. 1	32.75		
Coker-Cleveland 884-4 and Acala 4067	137.70		
Coker-Cleveland 884-4 and Rowden 40	37.57		
Farm Relief No. 1 and Acala 4067	92.79		
Farm Relief No. 1 and Rowden 40	51.41		
Acala 4067 and Rowden 40	131.49		

[1] $n'=6$ regions or classes. Then n for value of P in Elderton's table (where $n'=5+1=6$) equals 5. (See ELDERTON, W. PALIN. TABLES FOR TESTING THE GOODNESS OF FIT OF THEORY TO OBSERVATION. Biometrika 1:[155]-163. 1901.) One degree of freedom was used in the classification for the 6 regions.

SIMPLE CORRELATIONS WITHIN VARIETIES

From the data in columns 2 to 7, lines 1 to 10, of tables 2, 3, 4, 5, 6, and 7 simple correlation values were calculated to show the relationship of density of fiber population, fiber length, fiber weight, fiber strength, percentage of thin-walled fibers, and fiber diameter in the Mexican 128 variety of cotton. Calculations were also made from similar data for the other four varieties to obtain this relationship. The correlation coefficients for the five varieties are presented in table 40.

[10] LOVE, HARRY H. APPLICATION OF STATISTICAL METHODS TO AGRICULTURAL RESEARCH. 501 pp., illus. Shanghai. 1937. (See pp. 293-296.)

The values in table 40 are not significant for the correlation of density of fiber population with fiber length; whereas all the values for the correlation of density of fiber population with fiber weight are highly significant and negative—that is, as fiber population increases fiber weight decreases, or vice versa. The correlation of density of fiber population with the average strength per fiber is also negative and highly significant for each variety. The correlation values indicate a highly significant and positive relationship of density of fiber population with the percentage of thin-walled fibers in each variety. No variety shows a significant value for the relation of density of fiber population to the average fiber diameter.

TABLE 40.—*The simple correlation within varieties of density of fiber population, fiber length, fiber weight, fiber strength, percentage of thin-walled fibers, and fiber diameter for all five varieties of cotton.*

Variables paired	Simple correlation coefficients for variety named [1]				
	Mexican 128	Coker-Cleveland 884–4	Farm Relief No. 1	Acala 4067	Rowden 40
Density of fiber population and average fiber length	0. 0780	0. 1164	−0. 1528	0. 2200	0. 2354
Density of fiber population and average fiber weight per inch	**−. 6745	**−. 7089	**−. 6800	**−. 6978	**−. 5413
Density of fiber population and average strength per fiber	**−. 5582	**−. 7152	**−. 6771	**−. 6215	**−. 4270
Density of fiber population and percentage of thin-walled fibers	**. 7088	**. 6361	**. 7830	**. 7741	**. 6406
Density of fiber population and average fiber diameter	−. 1083	−. 0741	. 2288	. 0826	. 2439
Average fiber length and average fiber weight per inch	**−. 4106	**−. 3853	−. 1562	**−. 4443	−. 2495
Average fiber length and average fiber strength	−. 0732	−. 0379	. 0339	*−. 2548	−. 0758
Average fiber length and percentage of thin-walled fibers	. 1076	. 1338	. 0454	**. 3467	*. 2876
Average fiber length and average fiber diameter	**−. 3433	−. 1894	−. 1702	. 0705	. 1612
Average fiber weight per inch and average fiber strength	**. 6281	**. 7922	**. 5728	**. 7972	**. 3832
Average fiber weight per inch and percentage of thin-walled fibers	**−. 5919	**−. 7202	**−. 7928	**−. 7725	**−. 4416
Average fiber weight per inch and average fiber diameter	**. 3561	. 2006	−. 0743	. 1539	. 0985
Average fiber strength and percentage of thin-walled fibers	**−. 5831	**−. 7360	**−. 5754	**−. 7751	**−. 7717
Average fiber strength and average diameter	. 0856	. 1187	*−. 2955	−. 0636	−. 0144
Percentage of thin-walled fibers and average diameter	−. 0001	−. 0744	*. 2585	. 0169	*. 2740

[1] $n'=60$ regions. $n'-2=58$ degrees of freedom for each variety. *significant values; **highly significant values. Significance in this and all other correlation tables of this paper is based upon the required values given by WALLACE, H. A., and SNEDECOR, GEORGE W. CORRELATION AND MACHINE CALCULATION. Rev. by G. W. Snedecor. Iowa State Col. Off. Pub. 30, No. 4, 71 pp., illus. 1931. (See table 16.)

The correlation coefficients in table 40 for the relation of average fiber length to average fiber weight per inch are all negative, but are significant in only three varieties; namely, Mexican 128, Coker-Cleveland 884–4, and Acala 4067. The values show that the correlation of the average fiber length with the average fiber strength is significant in only one variety, Acala 4067, for which the value is also negative. The values for the relationship of average fiber length to the percentage of thin-walled fibers are all positive, but they are significant in only two varieties; namely, Alcala 4067 and Rowden 40. The correlation of average fiber length with average fiber diameter is significant in only one variety, Mexican 128, for which it is negative.

The coefficients for the relationship of average fiber weight and average fiber strength in table 40 are all positive and highly significant. The values for the correlation of fiber weight with the percentage of thin-walled fibers are negative and highly significant for each variety. The values indicate that the correlation of fiber weight with fiber diameter is significant in only one variety, Mexican 128, for which it is positive.

The values in table 40 show a highly significant and negative correlation of average fiber strength with the percentage of thin-walled fibers in each variety. The correlation of fiber strength and average fiber diameter is significant in only one variety, Farm Relief No. 1, in which the association is negative.

The coefficients given in table 40 for the relation of the percentage of thin-walled fibers to the average fiber diameter are significant in only two varieties, Farm Relief No. 1 and Rowden 40. The association is positive.

SIMPLE AND PARTIAL CORRELATIONS FOR THE ENTIRE EXPERIMENT

Simple and partial correlations of the fiber characters by regions on the cottonseed and also by plants, or seeds, are shown for the entire experiment in table 41.

Correlations by Regions

The correlation coefficients calculated for any character from the 300 regions on the 50 seeds of the five cotton varieties (6 regions$\times$50 seeds=300 regions) are based upon differences between regions within seeds and also between regions of seeds, or plants. The values in table 41 calculated from 300 regions are therefore not independent of the position of the fibers on the seed coat. The simple and partial correlations calculated from the data of 300 regions and shown in table 41 indicate the following relationships:

(1) The simple association of fiber population with average fiber length is not significant, while the partial association is significant and negative. As density of fiber population increases, fiber length decreases when the other characters, fiber weight, fiber strength, percentage of thin-walled fibers, and fiber diameter, are held constant. The simple and partial values for the relationship of fiber population with average fiber weight per inch, or unit weight, and also with average breaking load, or strength, per fiber are very significant and also negative. Positive and highly significant values are recorded for the simple and partial correlations of fiber population with the percentage of thin-walled fibers; that is, an increase in density of fiber population is associated with an increase in the percentage of thin-walled fibers. The simple and partial values for the association of fiber population with average fiber diameter are not significant.

(2) The simple and partial values indicate a negative and very real correlation of average fiber length with fiber weight per inch. The simple correlation of fiber length with fiber strength is significant with a negative value, while the partial relationship is not significant. Neither the simple nor the partial value for the relation of fiber length and the percentage of thin-walled fibers is significant. The coefficient for the simple correlation of fiber length and diameter is not considered significant, but the partial value for this relationship is positive and highly significant.

TABLE 41.—*Simple and partial correlations of density of fiber population, fiber length, fiber weight, fiber strength, percentage of thin-walled fibers, and fiber diameter for the entire experiment including all five varieties of cotton*

Simple order subscript [1]	Simple correlation coefficients [2]		Partial order subscript	Partial order coefficients	
	By regions ($n'=300$) [3]	By plants, or seeds ($n'=50$) [4]		By regions ($n'=300$) [3]	By plants, or seeds ($n'=50$) [4]
12	0.0515	*−0.2936	12.3456	*−0.1235	*−0.3071
13	**−.6312	*−.2827	13.2456	**−.3373	−.1895
14	**−.5662	−.2276	14.2356	**−.1798	−.1750
15	**.6601	.2643	15.2346	**.3661	.2081
16	−.0138	**−.4310	16.2345	.0437	−.2440
23	**−.2561	−.2455	23.1456	**−.3022	**−.4237
24	*−.1245	−.1604	24.1356	−.0221	−.2654
25	.0762	−.1006	25.1346	−.0710	−.1250
26	.0811	.1864	26.1345	**.1664	.2845
34	**.5874	.1730	34.1256	**.2600	−.0600
35	**−.5924	−.1143	35.1246	**−.2562	−.1330
36	**.1801	**.4544	36.1245	**.2975	**.4532
45	**−.5799	−.1860	45.1236	**−.2473	−.1730
46	.0311	.2105	46.1235	−.0017	.1800
56	.0824	−.0058	56.1234	**.2037	.1940

[1] 1=density of fiber population; 2=average fiber length; 3=average fiber weight per inch; 4=average breaking load per fiber; 5=percentage of thin-walled fibers; 6=average fiber diameter.

[2] *Significant value; **highly significant value.

[3] $n'=300$ regions. $n'-2=298$ degrees of freedom for each simple value. $n'-6=294$ degrees of freedom for each partial value.

[4] $n'=50$ plants, or seeds. $n'-2=48$ degrees of freedom for each simple value. $n'-6=44$ degrees of freedom for each partial value.

(3) The simple correlation of average fiber weight with average fiber strength is positive and very significant, and the same is true of the partial relationship. Negative and highly significant simple and partial coefficients are shown for the relation of fiber weight and the percentage of thin-walled fibers. Positive and very significant simple and partial values are noted for the correlation of fiber weight and average fiber diameter.

(4) Negative and highly significant simple and partial correlations are recorded for the association of average fiber strength and the percentage of thin-walled fibers. Simple and partial values for the relationship of fiber strength and fiber diameter are not considered significant.

(5) The value for the simple correlation of the percentage of thin-walled fibers with average fiber diameter is not noted as significant, while the partial value is positive and very real.

CORRELATIONS BY PLANTS, OR SEEDS

The mean value of any fiber character for a cottonseed, or plant, was obtained by summing the values of the six regions and then dividing the total by six. This mean value represents the average value of the six regions but does not necessarily represent the true average of any of the fiber characters on any one seed, or plant. The correlation coefficients in table 41 have been calculated from 50 plant, or seed, means for each character, and therefore the influence of position on the seed upon any fiber character has been eliminated in the correlation values. The calculations are based upon differences between plants within varieties and also upon differences between plants of different varieties, and not upon differences between regions, or positions. The simple and partial correlation coefficients calculated

from the mean values of 50 plants, or seeds, and presented in table 41 point to the following relationships:

(1) The association of fiber population and fiber length is indicated by significant and negative values for both the simple and the partial orders. The simple correlation value for the relation of fiber population to unit fiber weight is significant and negative, while the partial value is not indicated as significant. The simple and partial values for the association of fiber population with fiber strength and also with the percentage of thin-walled fibers do not appear to be significant. The simple correlation of fiber population and fiber diameter is highly significant and negative, but the partial value is not recorded as significant.

(2) The total correlation of fiber length with unit fiber weight is not significant, but a highly significant and negative value is indicated for the partial order. None of the simple or partial order values are significant for the correlation of fiber length with either fiber strength or percentage of thin-walled fibers. The simple association of fiber length and fiber diameter does not appear to be significant, and the partial value is not significant.

(3) The simple and partial values do not indicate a significant correlation of fiber weight with either fiber strength or percentage of thin-walled fibers. The association of fiber weight and fiber diameter is positive and very real in both the simple and the partial relationships.

(4) No significant relationship of fiber strength with either the percentage of thin-walled fibers or the fiber diameter is indicated by the simple and partial values.

(5) The simple and partial order values do not indicate a significant association of the percentage of thin-walled fibers and fiber diameter.

DISCUSSION

The results presented show that for each of the five varieties the population of region 1 (which includes the chalazal area at the basal end of the seed) is significantly denser than that of any other region. The mean fiber population for each of the six regions (see fig. 1) studied indicates that the varieties are somewhat similar in the distribution of fiber population, in that density is highest at the chalazal region and gradually becomes less as the raphe and the micropylar end of the seed are approached. The mean fiber population for regions, or punch areas, of the whole experiment indicates that the population of region 1 is significantly denser than that of any other region; that the population of region 3 is significantly denser than that of regions 4, 5, and 6; that the population of region 2 is significantly denser than that of regions 4 and 6; and that the population of region 5 is significantly denser than that of region 4. There is apparently a tendency for the fiber population to decrease in going from the chalazal area toward the micropylar, or apical, end of the seed; and the population also appears to decrease in a horizontal direction (see fig. 1) in going from regions 3, 5, and 6, toward the raphe. In order to compare these results with those of previous work, the fiber-population means per region, or punch area, in table 32 have been divided by the area per region, 3.567 $mm.^2$, and are presented in table 42, along with data previously obtained.

TABLE 42.—*The fiber distribution of the entire experiment as compared with that obtained from previous data*

OBSERVED VALUES

Region No.[1]	Source of data and average fiber population per square millimeter of seed-coat surface		
	Entire experiment consisting of 5 varieties (1937 crop)	20 naked [2] seeds from 20 plants (1924 crop) [3]	4 seeds [4] from 4 plants (1924 crop) [3]
1	469	175	210
2	117	57	81
3	142	64	116
4	71	32	47
5	98	43	78
6	81	16	49

VALUE

Kind of comparison	Value of χ^2	Value of P	Odds
Columns 2 and 3 from above	8.12	0.087847	10 to 1
Columns 2 and 4 from above	20.67	.000257	3,890 to 1

[1] $n'=6$ regions. Then n for value of P in Elderton's table (where $n'=5+1=6$) equals 5. 1 degree of freedom was used in the classification for the 6 regions . (See reference given in footnote to table 39, p. 293.)
[2] No fuzz on seeds and also a sparse fiber population.
[3] See footnote, 256.
[4] Fuzzy tip and base and sparse fuzz on middle part of seed. Normal fiber population.

The values for goodness of fit in table 42 show that there is fair agreement for the fiber distributions of the present results with the data obtained [11] on fuzzless seeds. The agreement is poor, however, when the present data are compared with results from seeds having dense fuzz on the tip and base and sparse fuzz on the middle portion of the seed coat. The data in columns 2, 3, and 4 show a similarity in the three sets of data; namely, in each case the population is densest on region 1 and tends gradually to decrease in areas toward the micropylar end and the raphe of the seed.

The average fiber length of regions for the whole experiment indicates that the length from basal regions (1, 2, and 3) is greater than the length of regions (4, 5, and 6) near the apical, or pointed, end of the seed. This agrees with the report made by Koshal and Ahmad [12] on Surat and Indian Standard cottons. They state that the mean fiber length at the base of the seed is significantly longer than that at the apex of the seed.

The average fiber weight by varieties and for the whole experiment shows that the weight per inch of fibers from region 1 is much lighter that that from any other region. The results from the same source also indicate a lower fiber weight for the basal area (regions 1, 2, and 3) than for the area (regions 3, 4, and 5) near the apex, or tip, of the seed. Such conclusions are also similar to those published by Koshal and Ahmad, who report that the mean fiber weight per unit of length is significantly higher for the apical than for the basal fibers. Campbell [13] also found that the percentage of undeveloped fibers (having thin walls and relatively light weight) is highest at the basal end of the seed, becoming lower as the pointed end is approached.

[11] See footnote 2.
[12] See footnote 4.
[13] See footnote 3.

Photomicrographs showing unstained transections of mature cotton fibers from regions 1 and 6 of a seed from one of the cotton varieties studied: *A*, Relatively thin fiber walls from region 1, which includes the chalazal area; *B*, relatively thick fiber walls from region 6, near the micropylar end. × 528.

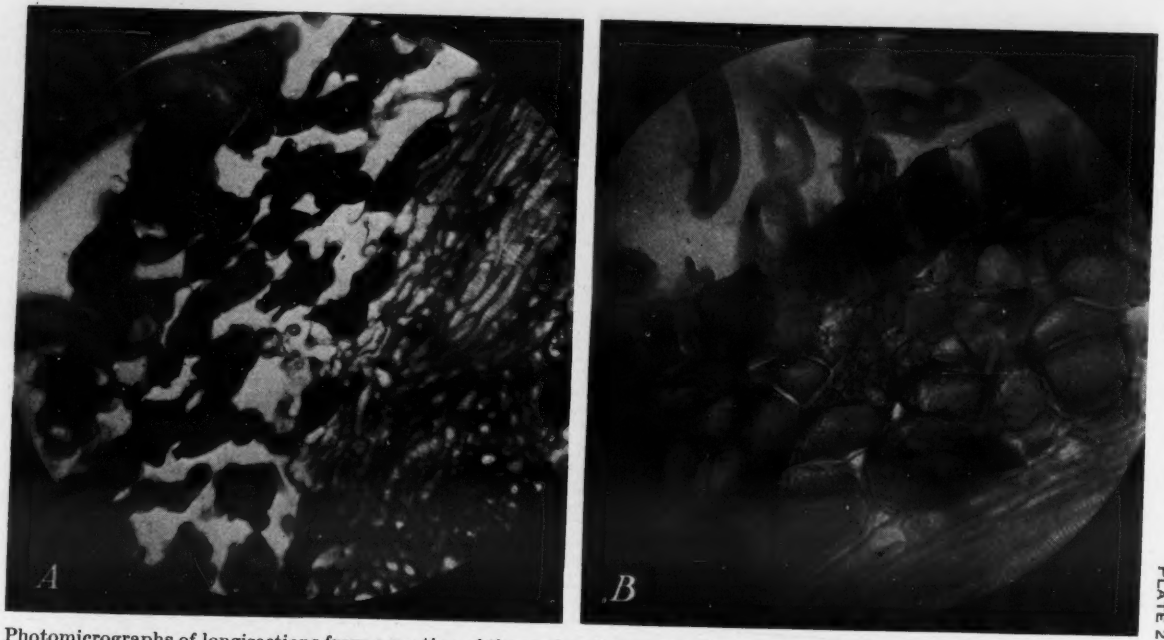

PLATE 2

Photomicrographs of longisections from a portion of the seed coat from a mature seed of one of the cotton varieties studied: *A*, From chalazal area; shows a relatively large amount of intercellular space between the vein and the epidermal layer, to which are attached portions of three cotton hairs. *B*, From approximately region 3 and some distance from the chalaza; shows a relatively small amount of intercellular space between the vein and the epidermal layer, to which are attached portions of two cotton hairs; parts of hairs which are not attached to the epidermal layer and cut at various angles are also shown. × 316.

Within each of the five varieties and for the entire experiment, the average breaking load of fibers from the basal part of the seed is significantly lower than that of fibers from the apical portion of the seed. Within varieties (with the exception of Rowden 40) and for the whole experiment, the average breaking load of fibers from region 1 is significantly less than that of any other region. Similar results are reported by Koshal and Ahmad, who measured the breaking load of single fibers at and near the chalazal region and also at the apical end of the seed and who state that the mean fiber strength is significantly higher for the apical than for the basal fibers in Surat and Indian Standard cottons.

Within varieties and also for the entire experiment the percentage of thin-walled fibers is much higher on region 1 than on any other region. For the entire experiment the percentage of thin-walled fibers is significantly higher on the basal end of the seed than on the apical end; and within varieties the condition is similar. Transections of mature cotton fibers from regions 1 and 6 of a cotton seed are shown in plate 1. It will be noted that the thin-walled fibers are predominant in region 1 and very scarce in region 6. Campbell has already been quoted in the discussion of fiber weight.

Significant differences in fiber diameter between regions on the seed coat within varieties are recorded for only two varieties, Acala 4067 and Mexican 128. For the entire experiment the results indicate no significant differences in the average fiber diameter between regions on the seed coat. The detailed records on file point to the fact that variability in the fiber diameter of the five cotton varieties is due mostly to variability within regions.

The distribution of each of the six fiber characters on the cottonseed has been discussed and compared with previous data, but no reasons have been advanced to explain the distributions. Why is the fiber population denser on the basal surface of the seed, especially in the chalazal area, than on surfaces nearer the raphe and the tip, or micropylar end? The fiber population on the seed coat of naked seed and other types was previously determined by the author, who also made studies relative to the venation of the seed coat. A conclusion was reached that the venation of the seed coat was relatively sparse in the areas bordering each side of the tip half of the raphe and that a relatively low fiber population occurred in such areas. The venation pattern may be related to the supply of food and thus to the variability of density of population; but, since density of population and unit weight are inversely correlated, there is not much, if any, evidence for the first relationship. The fiber population in American upland cotton types is determined within about 3 days (counting the date of flowering as the first day); the maximum fiber diameter is probably attained by the third day, and the full fiber length is reached in from 17 to 20 days, after which growth of the secondary wall begins and continues for an indefinite number of days until it is completed. It does not seem that differences in the available food supply would affect the density of fiber population, since it is likely that there is sufficient food available at any location on the seed coat for the production of hair initials. Another question might also be asked. Why are the longer, weaker, and lighter fibers (per unit of length) with a relatively high percentage of thin walls on the basal surface of the seed? The available food supply as affected by variation in venation

does not answer this question. The relatively shorter fibers near the tip end of the seed are also heavier per unit of length, and it is probable that much more food is used for cell thickening than for elongation. Since the fibers at and near the apex of the seed coat are heavier, they also have thicker cellulose walls and are stronger.

It is conceivable that the very low relative fiber weight of region 1, which includes the chalazal area, is related to the loose arrangement of cells under the epidermal layer. That is, there is an appreciable area of space between the cells (pl. 2), and as a result transportation of foods to the chalazal area may be adversely affected. Since this region also has a high population, the limited food supply may not be sufficient for the normal wall development of all the fibers. Such a condition could be responsible for the relatively low fiber weight, relatively low fiber strength, and relatively high percentage of thin-walled fibers of region 1. No satisfactory explanation can be given to answer the two questions that have been raised. It is probable that inherent factors and physiology both play an important role in determining the distribution of fiber population, length, unit weight, strength, percentage of thin-walled fibers, and diameter.

Differences between plants and also between varieties in the distribution of fiber population, length, unit weight, strength, percentage of thin-walled fibers, and diameter are probably inherent.

SUMMARY AND CONCLUSIONS

The distribution and relation of fiber population, length, breaking load, weight, diameter, and percentage of thin-walled fibers on the cottonseed were measured in the 1937 crop of five varieties of American upland cotton (*Gossypium hirsutum* L.). Six regions, or punch areas, numbered one to six, were studied on each seed. Ten seeds from ten plants of each variety were investigated.

Analysis of variance was applied to the data for each variety and also to the data for the entire experiment. Simple correlation coefficients were calculated to show the relationship of the six fiber characters one to another within varieties, and both simple and partial correlation values were obtained to indicate their relationship for the entire experiment by regions, or punch areas, and also by seeds, or plants.

The fiber population is densest at and near the chalazal area of the seed and becomes thinner downward toward the micropylar end and outward toward the raphe. Goodness of fit between the five varieties for the fiber population of the six regions shows that no two varieties are alike in the fiber distribution.

The results from the whole experiment indicate that fibers on the basal end of the seed are lighter, thinner, weaker, and longer than those on the apical or micropylar end.

Varieties significantly affect the population, diameter, and percentage of thin-walled fibers of regions.

There are real differences between varieties in the means of fiber population, length, breaking load, diameter, and percentage of thin-walled fibers.

The results obtained should serve as stepping stones to further progress in cotton-breeding and production work. Plants (as represented by one seed) within a variety show differences among themselves relative to fiber length, unit fiber weight, fiber strength, fiber

diameter, and percentage of thin-walled fibers—that is, regional differences. Some plants are more uniform than others. In the five varieties studied, however, it seems that the variation of fiber length and diameter on the seed is due mostly to variability within regions. This indicates that selection of plants for uniformity of length and diameter should be largely on the basis of uniformity of the whole fiber population of the seed without especial regard to regional differences. It appears that the breeder may be able to select plants which show a relatively low amount of variation in fiber weight, fiber strength, and percentage of thin-walled fibers between regions on the seed and thus eliminate some of the variability in these properties.

The present paper suggests possibilities in cotton improvement work; yet it, along with records on file, also indicates that there is probably considerable variation between regions in the fiber population, fiber length, unit fiber weight, fiber strength, and percentage of thin-walled fibers on single seeds of the five improved cotton varieties, indicating that the uniformity of a variety in any of these characters is limited by the variability found on the seed. Significant differences between varieties in the distribution and means of fiber population, length, strength, percentage of thin-walled fibers, and diameter are probably inherent. Differences between varieties in fiber length are of some value in varietal classification, and it is conceivable that differences between them in the distribution of fiber population may help in taxonomic classification.

The simple correlation within varieties of density of fiber population with average fiber weight per inch, with average strength per fiber, and with the percentage of thin-walled fibers is very significant and striking in each variety; and an increasing fiber population is thus associated with a decreasing fiber weight and fiber strength and with an increasing percentage of thin-walled fibers. Within each variety the simple correlations indicate a very consistent association of average fiber weight per inch with average strength per fiber and also with the average percentage of thin-walled fibers. An increasing fiber weight is correlated with an increasing fiber strength and with a decreasing percentage of thin-walled fibers. All these relationships, although not independent of the position of the fibers on the seed and the other fiber characters involved, appear to have some significance. For instance, selection for a denser fiber population should also lower both the fiber weight and the fiber strength and should raise the percentage of thin-walled fibers. Selection for a higher fiber weight per inch should raise the fiber strength and lower the percentage of thin-walled fibers. The simple correlation of average strength per fiber with the percentage of thin-walled fibers is very consistent and significant within each variety, where an increasing fiber strength is associated with a decreasing percentage of thin-walled fibers.

The simple and partial correlations by regions for the entire experiment of density of fiber population with average fiber weight per inch, with average strength per fiber, and with the percentage of thin-walled fibers are similar in each relationship to the simple values within varieties; that is, an increasing population is asosciated with a decreasing fiber weight and fiber strength and with an increasing percentage of thin-walled fibers. For the entire experiment by regions the total, or simple, correlation of fiber population with fiber length

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does not appear to be significant, while the partial value is significant and negative; that is, with fiber weight, strength, diameter, and the percentage of thin-walled fibers held constant, an increasing fiber population is associated with a decreasing fiber length. The partial relationships discussed in this paragraph are not independent of the position of fibers upon the seed; however, it appears that selection on the cottonseed for a higher population should decrease fiber length, weight, and strength and increase the percentage of thin-walled fibers. For the whole experiment by regions there is a real negative association of average fiber length with average fiber weight per inch in both the total and the partial orders; the former fiber character shows a significant and negative simple association with average strength per fiber, but the partial value is very small and not significant; and independent of all other fiber characters there is perhaps a positive association of fiber length with average fiber diameter. Raising the fiber length should therefore theoretically increase fiber diameter and lower fiber weight. For the entire experiment by regions average fiber weight per inch appears to be correlated with average strength per fiber, with the percentage of thin-walled fibers, and with average fiber diameter in both the simple and the partial orders; that is, as fiber weight goes up, fiber strength and diameter increase and the percentage of thin-walled fibers decreases. As average strength per fiber for the whole experiment by regions increases, the percentage of thin-walled fibers goes down in both the simple and the partial orders; and an increase in the percentage of thin-walled fibers is associated with a larger fiber diameter in the partial relationship.

In a general way the total and partial-order correlations for the complete experiment by plants, or seeds, are similar to those for the entire experiment by regions, expecially as to signs; but relatively few of the values are significant. This might be expected since there are only 50 plants, whereas there are 300 regions. For the entire experiment by plants (where each plant, or seed, is an average of the 6 regions and where the differences are between plants and not between regions, or position) density of fiber population is negatively associated with average fiber length in both the simple and the partial orders, and a negative relationship is also noted for the simple association of fiber population with average fiber weight and with average fiber diameter. The entire experiment by plants indicates that independent of all other fiber characters, average fiber length is negatively associated with average fiber weight per inch and that average fiber weight is positively associated with average fiber diameter. Selection of plants, or seeds, for higher population should lower the fiber length; selection of plants for longer fiber length should lower fiber weight; and selection for higher fiber weight should raise fiber diameter.

RELATION OF DIET OF SWINE TO DEVELOPMENT OF LOCOMOTOR INCOORDINATION RESULTING FROM NERVE DEGENERATION [1]

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INTRODUCTION

A troublesome locomotor disorder among pigs, manifested by incoordination in the use of the legs, abnormal posture, and lameness, which has been of common occurrence in the Bureau's herd at the United States Department of Agriculture, Beltsville Research Center, Beltsville, Md., was described in an earlier paper (*1*).[2] The observations there reported suggested both diet and breeding as possible causative factors, particularly among pigs confined in small pens and fed in record-of-performance tests. The role of breeding was suggested by the high incidence of lameness among inbred litters, and some significance was placed on the abnormally low free-choice intake of calcium- and phosphorus-rich components of the diet.

Although a lack of either vitamin A or vitamin D is recognized as causing characteristic types of lameness and paralysis, the disorder apparently persisted under a regimen considered adequate in these vitamins for growing and fattening pigs. It appeared that if the disease were of dietary origin, the cause was possibly an obscure derangement of calcium and phosphorus metabolism or suboptimum levels of an unknown vitamin or vitaminlike factor in the stock diet. An infection of swine erysipelas in the herd was also considered as a possible complicating factor since a small proportion of the growing pigs had swollen joints and other symptoms of this disease, which also occasionally resulted in lameness. However, no consistent gross lesions of the bones or joints, other than some possibly due to injury, were detected in the animals suffering from the type of incoordination under investigation.

Further study of the problem was accordingly undertaken along the following lines: Determination of the effects of mineral elements in the diet; search for easily available feeds for use as supplements or replacements in the stock diet which would completely prevent or cure the disease; and, in order to facilitate the identification of the deficiency factor, the formulation of an experimental diet or diets capable of producing the symptoms. Control of the lines of breeding of the animals used in experimental lots and of the incidence of erysipelas was coordinated with the dietary treatments. The results obtained are reported in this paper.

REVIEW OF LITERATURE

A disease characterized by posterior paralysis and incoordination of movement was described by Wehrbein (*7*) of the Iowa State College

[1] Received for publication July 11, 1940.
[2] Italic numbers in parenthesis refer to Literature Cited, p. 316.

of Agriculture in 1916. Degeneration of the myelin sheath and of the brachial and sciatic nerves was observed. Efforts to demonstrate pathogenic organisms failed. In more recent years, Eveleth and Biester (*2*), also of the Iowa State College, have continued the work and have reviewed the reports of other investigators from the standpoint of the possible involvement of vitamin A and of the vitamin B complex in the nerve degeneration in various species. The complexity of the problem is evidenced by the conclusions of the Iowa investigators, which indicate that the incoordination and myelin degeneration in the nervous systems of swine are not necessarily associated in all cases and that neither vitamin A nor the vitamin B complex is responsible for the myelin degeneration.

In work on young pigs fed artificial diets, Wintrobe, Mitchell, and Kolb (*7*) observed poor growth, marked ataxia, and severe nerve and spinal-cord degeneration when the content of yeast was gradually reduced and thiamin and riboflavin substituted in the ration. In work at the Beltsville Research Center (*6*) on the vitamin B_1 requirement of young swine, nerve and spinal-cord degeneration have also been encountered in animals fed autoclaved diets with and without the addition of thiamin.

The observations of Hogan and coworkers (*3*) are also of interest because of their relation to sows and suckling pigs and because the development of the abnormal gait seemed to depend on sudden changes in the nutritive value of one or more constituents of the diet. These investigators reported serious losses during certain years in the pig crop, whereas in others the disease was mild in form or absent.

Hughes (*4*) has worked with purified diets in which the components of the vitamin B complex were varied. He observed marked lameness in pigs fed a diet low in riboflavin, although other groups on riboflavin-rich diets that were in turn low in thiamin, nicotinic acid, or other fractions of the B complex were not free of lame pigs.

Besides these reports, information in the possession of the Bureau, including communications from a number of workers at various State experiment stations, indicates that the disorder is of serious economic importance and is more prevalent in some years than in others.

EXPERIMENTAL PROCEDURE

Beginning with the spring farrow of 1937 and continuing through the spring farrow of 1939, groups of pigs were fed in concrete-floored pens under essentially the same conditions as those prevailing in record-of-performance tests except for dietary differences. Two experiments, one with spring and the other with fall pigs, were conducted each year. In the earlier experiments, the number of lots was restricted to six, and litters containing at least six pigs were utilized in order to provide litter mates in each lot. These litters were selected so far as possible from inbred stock which had shown a predisposition toward lameness, in an attempt to accentuate the effects of the various diets either in completely protecting against or in intensifying the symptoms. After 1937, this inbred stock became so depleted in the herd that it was not possible to continue this procedure. In the more recent experiments, it has been necessary also to depart somewhat from the use of complete litter-mate groups and

to select closely related pigs of similar age, sex, and weight. The pigs were generally placed on experiment at 10 to 12 weeks of age.

The diets were prepared from the general supply of feeds purchased for use in swine feeding at Beltsville. The stock diet consisted of No. 2 yellow corn, digester tankage of 60 percent protein content, linseed meal, alfalfa-leaf meal made from sun-cured hay, and a mineral mixture. This mineral mixture consisted of ground limestone, 50 percent; steamed bonemeal, 27.97 percent; common salt, 20 percent; iron oxide, 2 percent; potassium iodide, 0.02 percent; and copper sulfate, 0.01 percent. This mixture constituted 0.8 to 1.0 percent of the stock diet. The supplemental or replacement feeds were varied, being chosen because of known or supposed mineral, vitamin, or protein value and as contrasting feeds for replacement of the corn, tankage, linseed meal, or alfalfa-leaf meal.

The diets were adjusted, through changes in quantities of corn or other cereal and tankage, or its substitute, to furnish approximately 18 percent of protein to pigs between the weights of 40 and 100 pounds, 15 percent to those between the weights of 100 and 160 pounds, and 12 percent to those with weights of more than 160 pounds. Whenever linseed and alfalfa-leaf meals were used, they generally constituted 5 percent of the diet of the pigs in the lowest weight group, 4 percent of that of the middle group, and 3 percent of that of the highest weight group.

The diets fed were grouped in five series and are shown in table 1. In series A, particular attention was given to the effect of minerals on the production of lameness. As shown in the table, group 2 received a low mineral diet, and group 8 received a supplement of manganese chloride. Oats were used in group 6 in part because of their relatively high manganese content. Group 5 was given access to a small unpaved area of natural clay soil free of vegetation to determine whether the lacking element or elements in the diet could be obtained from the soil. Skim milk, green forage, and liver were fed in part for their mineral content but more in a search for a natural feed that would be consistently protective against the disease. The green forage consisted of both freshly cut green soybeans and alfalfa. It was estimated that the quantity fed corresponded to approximately 5 percent of the dry forage in the diet.

In series B, the use of corn-gluten meal in place of tankage and alfalfa-leaf and linseed meals was prompted by the deficiency of the first-named feed in riboflavin, as indicated by early assays for vitamin G, and the desire to determine the possible role of riboflavin in the prevention of the disease. Oats were used in group 2 because of the favorable results obtained in series A. Liver was used in the next group for its high riboflavin content and also for its richness in other members of the vitamin B complex. In one group molasses was also used for its richness in certain factors, including vitamin B_6. Casein, washed with acidulated water and extracted with alcohol, was added in one group to improve the quality of protein mixture and as a control on the other diets to which natural feeds were added. Fortified cod-liver oil was added to the diets of series B, as well as to those of series C and D, in order to provide an adequate supply of vitamins A and D in all rations regardless of composition and treatment.

TABLE 1.—*Experimental set-up for the five series of tests*

Pig group No.	Trials	Pigs used in test	Proportion of basal mixture used	Diet supplement or treatment
	Number		*Percent*	
SERIES A—BASAL MIXTURE OF CORN, TANKAGE, ALFALFA-LEAF MEAL, AND LINSEED MEAL				
1	2	Spring and fall	99.2	Mineral mixture, 0.8 percent (stock control diet).
2	2	do	100	None.
3	2	do	100	5 pounds of skim milk per day.
4	3	Spring	100	1 pound of green forage per day.[1]
5	1	do	99.2	Same as group 1; pigs on earth runway.
6	2	Spring and fall	70	Oats, 30 percent.
7	1	Fall	97	Dried pork liver, 3 percent.
8	1	do	100	Manganese chloride, 72 p. p. m.
SERIES B—BASAL MIXTURE OF CORN, CORN-GLUTEN MEAL, MINERAL MIXTURE, AND FORTIFIED COD-LIVER OIL				
1	1	Spring	100	None.
2	1	do	70	Oats, 30 percent.
3	1	do	98	Dried pork liver, 2 percent.
4	1	do	95.5	Casein, 4.5 percent.
5	1	do	85	Molasses, 15 percent.
SERIES C—BASAL MIXTURE OF TANKAGE, MINERAL MIXTURE, AND FORTIFIED COD-LIVER OIL				
1	2	Spring and fall	13.1	Corn, 86.9 percent.
2	2	do	13.1	Barley, 86.9 percent.
3	2	do	13.1	Wheat, 86.9 percent.
SERIES D—SIMPLE BASAL DIET OF CORN, TANKAGE, MINERAL MIXTURE, AND FORTIFIED COD-LIVER OIL OR STOCK CONTROL DIET OF ALFALFA-LEAF MEAL AND LINSEED MEAL ADDED TO SIMPLE BASAL DIET				
1	3	2 spring and 1 fall	100	Simple, unheated (control).
2	3	do	100	Stock, unheated (control).
3	2	Spring and fall	100	Simple, heated.
4	1	Spring	100	Stock, heated.
				Simple, heated, supplemented by—
5	1	Fall	90	Corn-gluten meal, 10 percent.
6	1	do	97	Dry liver, 3 percent.
7	1	do	90	Rice bran, 10 percent.
8	1	do	92.3	Dried whey, 7.7 percent.
				Stock, heated, supplemented by—
9	1	Spring	85.3	Dried whey, 14.7 percent.
10	1	do	85.3	Whey concentrate, 14.7 percent.
11	1	do	99	Wheat-germ oil, 1 percent.
SERIES E—CURATIVE TRIALS ON VARIOUS DIETS				
1	1	Fall	100	Stock control, same as series D, No. 2.
2	1	do	100	Dried skim milk in place of tankage in series D, No. 2.
3	1	do	100	Animals fed with groups 1, 2, 6, 7, and 8 in series A.

[1] Alfalfa-leaf meal omitted from basal mixture.

In series C, comparison was made of corn, barley, and wheat, when constituting a high percentage of the diet, for the prevention of lameness.

In series D, the basal diet and a simplification of it, in which the linseed and alfalfa-leaf meals were omitted, were subjected to dry

heat at 115°–120° C. for 40 hours in the case of the former diet and 30 hours in the latter. Poultry-nutrition studies have shown that a factor or factors essential for the nutrition of the chick are destroyed by the heat treatment. The dermatitis produced by the feeding of a heated diet has been cured or prevented by the feeding of the vitamin B complex fraction designated as the filtrate fraction, and recent work has been reported (*5*) on the protective properties of pantothenic acid. Apparently, thiamin, riboflavin, vitamin B_6, and possibly nicotinic acid are not inactivated or destroyed by the heat treatment. At the time that the first trials on the feeding of heated diets were planned (1938), it seemed evident that the factors just named were also not involved in the lameness derangement in pigs. In series D, as indicated in table 1, various supplements were used with the heated diets. The whey concentrate used in group 10 was prepared in the laboratories of the Bureau of Dairy Industry. In obtaining this product, whey from rennet-coagulated curd was evaporated and approximately two-thirds of the lactose allowed to crystallize out. Cod-liver oil was added to all diets at the time that new supplies of feed were placed in the self-feeders.

In series E, three groups of pigs are included that were segregated from the herd or from record-of-performance lots after definite signs of lameness had developed and were fed in an endeavor to determine whether cures or alleviation of symptoms could be obtained. One group received the stock diet, another a modified stock diet in which dried skim milk replaced the tankage, and the third consisted of a litter of five pigs that had been distributed among 5 lots in series A.

The pigs were kept on the test diets for periods of 12 to 24 weeks. As the experiments progressed, it was found that symptoms of lameness and incoordination were generally easily detected within a 12-week feeding period; consequently, the test period was shortened in later experiments. The mixed diets were self-fed and the consumption of each lot recorded. The pigs were weighed and examined weekly. A scoring system was set up by which to record the relative severity of the disease. Entire absence of symptoms was scored as 0; initial signs of lameness, as 1; more advanced lameness with tendency to weakness in pasterns and peculiar motion of rear legs, as 2 (fig. 1, *A*); definite incoordination shown by weaving motion and the throwing forward of the rear legs, also difficulty in rising and tendency to sit on the haunches, as 3; marked symptoms of those enumerated as 3, in which the animal had great difficulty in walking and frequently collapsed, as 4; and extreme paralysis, in which the animal was barely able to rise or to move about with the forelegs, as 5 (fig. 1, *B*).

At the conclusion of the feeding period, the animals from most of the groups were slaughtered for laboratory studies. Samples taken included blood for erysipelas tests, (made by the Pathological Division of the Bureau); also nerve and spinal cord tissue and the femur bones.

A histological study of the sciatic nerve and spinal cord of approximately 95 animals was made. Several peripheral nerves of the first few animals studied were sectioned for examination, but in the later work only the sciatic nerve and the spinal cord were taken for routine study since these tissues show the degeneration consistently when the other nerves are involved. The samples of nerve tissue were removed from the warm carcasses immediately after slaughter, and sections were

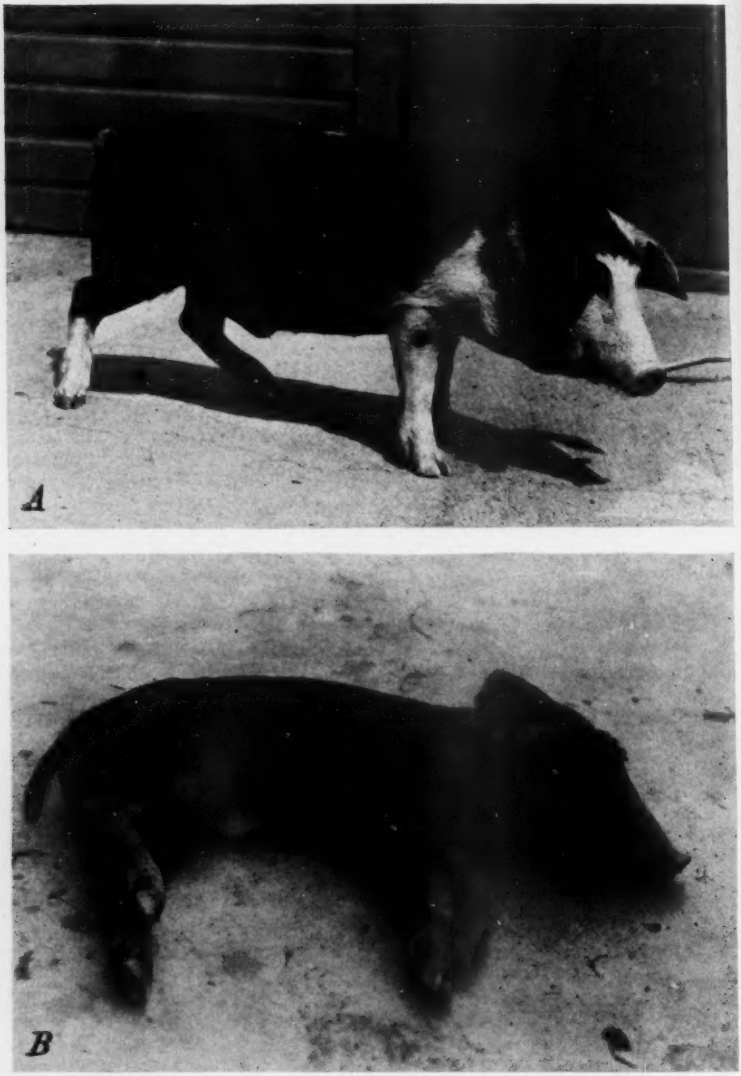
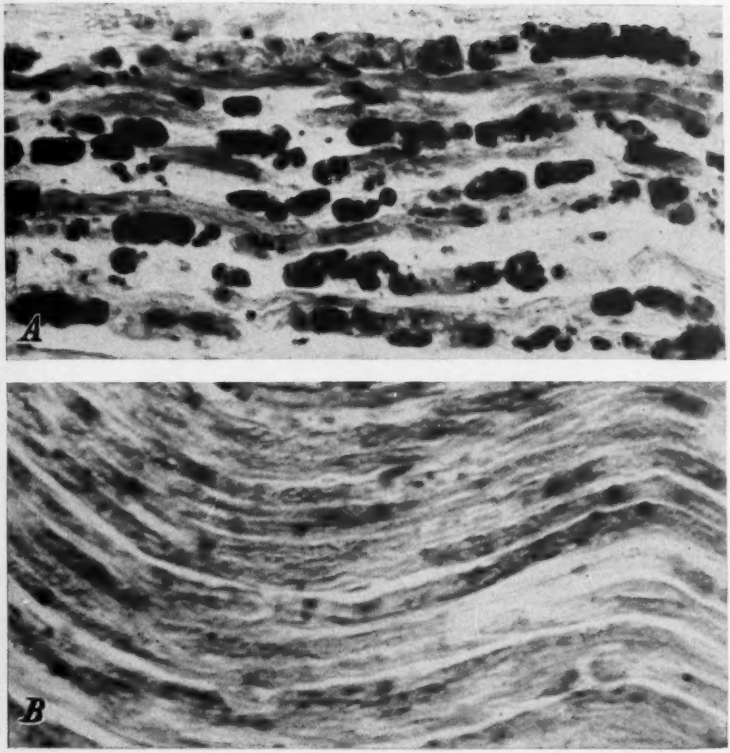
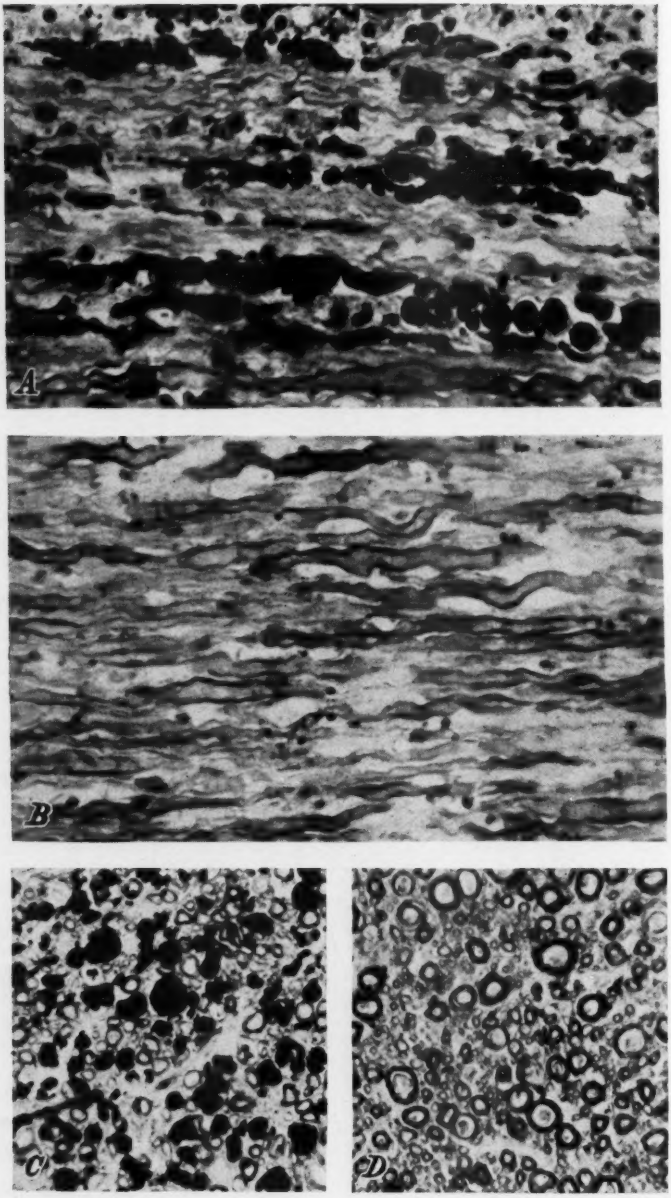


FIGURE 1.—*A*, Pig showing incoordination in the use of the hind legs. *B*, Pig with extreme paralysis, which developed while the animal was fed the heated diet No. 3 in series D.

prepared for study by the Marchi method. This procedure consists in placing the fresh tissues in a solution of potassium dichromate until the normal myelin is largely oxidized and then transferring to a water solution of osmium tetroxide and potassium dichromate to oxidize



A, Longitudinal section of the sciatic nerve from a typical animal with advanced locomotor incoordination showing massive demyelination of nerve fibers (black globular areas); *B*, longitudinal section of the sciatic nerve from a normal animal showing intact myelinated fibers.



A, Longitudinal section of a dorsal column of spinal cord from a partially paralyzed pig showing degeneration of myelinated tracts; *B*, longitudinal section of a dorsal column of the spinal cord from a normal animal showing normal medulated fibers; *C*, cross section of *A*; *D*, cross section of *B*.

further the degenerated myelin and ordinary adipose tissue. Oxidation of the products of the degenerated myelin sheaths results in a deposition in these areas of a lower oxide from reduced osmium tetroxide, which is black in color. The tissues were embedded in celloidin or paraffin, then cut and mounted in the usual way. The slides were either counterstained or examined directly for myelin degeneration, as shown by the globular black areas in plates 1 and 2. The absence, presence, and severity of myelin degeneration were scored on a numerical basis paralleling the method of scoring the live animals for lameness.

Supplementary to the hog-feeding experiments, a number of rat-feeding comparisons were made to determine the possible dietary deficiencies of the heated diets from the standpoint of rate of growth and feed utilization.

EXPERIMENTAL RESULTS

GROWTH OF PIGS AND INCIDENCE OF LAMENESS

Table 2 shows the results obtained with the pigs on the various series of diets. In series A, the animals in all eight groups grew at normal rates and remained on the diets until most of them weighed more than 200 pounds. As shown in the table, every group contained at least 1 affected animal. Of a total of 77 animals, 27 showed signs of lameness and incoordination. To a large extent, the cases were of a mild form. Perhaps the most striking result was the high incidence among the pigs fed green forage. The animals of this group (No. 4), like those of group 2, did not receive a mineral mixture, and the affected animals in these two groups showed a greater degree of lameness than those in any of the other groups.

Oats and dried liver were reasonably effective supplements. The former was fed as 30 percent of the diet, which is considered near the maximum for the most efficient utilization.

Most of the animals used in series A consisted of litters from the spring and fall farrows of 1937, many of which were selected from the inbred stock suspected as being predisposed toward the development of lameness. The data indicate that little was gained from the use of such stock to accentuate the effects of ordinary diets on the incidence and severity of incoordination. This finding has been confirmed by subsequent results with pigs representing a large number of lines of breeding and showing a varying incidence of incoordination from season to season.

In series B, the results obtained show rather definitely that the use of corn-gluten meal did not render the diets less effective in preventing lameness and incoordination. No affected pigs were found in either the control group or the group receiving oats, and only one mild case was recorded in each of the remaining groups. Growth on the control diet was poor. Although the rates of gain were highest with the feeding of liver and of casein, growth was still subnormal. Oats increased gains somewhat and molasses only slightly.

In series C, the three groups, regardless of the supplement fed, had similar rates of gain and final weights. The lowest incidence, as well as degree, of lameness was observed in the group receiving the barley supplement.

TABLE 2.—*Growth and incidence of lameness of groups of pigs on the five series of diets*

SERIES A.—BASAL MIXTURE OF CORN, TANKAGE, ALFALFA-LEAF MEAL, AND LINSEED MEAL

Pig group No.	Pigs	Diet supplement or treatment	Average weight		Average daily gain	Feed per 100 pounds of gain	Lame animals	Average score for lameness
			Initial	Final				
	Number		*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Number*	
1	11	Mineral mixture; control diet	43.6	204.6	1.50	370.9	3	0.67
2	11	None	48.6	214.1	1.72	356.4	5	.82
3	10	Skim milk	43.5	206.6	1.72	344.3	3	.50
4	18	Green forage	56.8	198.8	1.46	425.8	11	.98
5	5	Same as group 1; pigs on earth runway	39.4	229.8	1.72	357.0	1	.10
6	10	Oats	46.8	219.7	1.68	381.7	1	.10
7	6	Liver	54.7	213.9	1.63	384.8	1	.10
8	6	Manganese	54.7	218.7	1.82	348.3	2	.25

SERIES B.—BASAL MIXTURE OF CORN, CORN-GLUTEN MEAL, MINERAL MIXTURE, AND FORTIFIED COD-LIVER OIL

1	5	None	55.8	132.4	0.50	543.4	0	0.0
2	5	Oats	55.6	138.6	.75	467.3	0	.0
3	5	Liver	57.0	269.2	1.11	376.3	1	.2
4	5	Casein	61.3	171.1	.93	438.1	1	.17
5	5	Molasses	56.8	152.8	.58	569.2	1	.20

SERIES C.—BASAL MIXTURE OF TANKAGE, MINERAL MIXTURE, AND FORTIFIED COD-LIVER OIL

1	12	Corn	67.8	208.8	1.52	369.8	8	0.86
2	13	Barley	81.4	208.2	1.63	388.5	1	.04
3	13	Wheat	81.3	208.2	1.59	370.8	7	.42

SERIES D.—SIMPLE BASAL DIET OF CORN, TANKAGE, MINERAL MIXTURE, AND FORTIFIED COD-LIVER OIL OR STOCK CONTROL DIET OF ALFALFA-LEAF MEAL AND LINSEED MEAL ADDED TO SIMPLE BASAL DIET

1	17	Simple, unheated (control)	63.8	211.8	1.54	363.7	11	0.90
2	17	Stock, unheated (control)	62.8	230.3	1.78	359.6	6	.41
3	12	Simple, heated	53.6	115.6	.52	451.7	12	2.59
4	6	Stock, heated	65.8	66.2	0	[1]	6	2.25
		Simple, heated, supplemented by—						
5	6	Corn-gluten meal, 10 percent	57.7	107.7	.48	402.7	3	1.00
6	6	Dry liver, 3 percent	56.8	207.1	1.52	336.1	2	.75
7	6	Rice bran, 10 percent	58.5	151.7	.93	397.5	2	.58
8	6	Dried whey, 7.7 percent	60.0	202.0	1.41	353.2	2	.67
		Stock, heated, supplemented by—						
9	6	Dried whey, 14.7 percent	77.0	182.5	1.21	408.4	1	.33
10	6	Whey concentrate, 14.7 percent	74.7	162.7	.99	488.8	3	.67
11	6	Wheat-germ oil, 1 percent	70.7	98.5	.31	810.2	6	1.75

SERIES E.—CURATIVE TRIALS ON VARIOUS DIETS

Pig group No.	Pigs	Diet	Average weight		Average daily gain	Feed per 100 pounds gain	Lameness scores	
			Initial	Final			Beginning	End
	Number		*Pounds*	*Pounds*	*Pounds*	*Pounds*		
1	5	Same as series D, No. 2	83.8	146.0	0.87	.....	2.6	2.5
2	5	Dried skim milk in place of tankage in series D, No. 2	109.2	204.2	1.64	383.6	2.2	3.3
3	5	Animals fed with groups 1, 2, 6, 7, 8 in series A	70.8	215.0	1.85	380.2	2.0	1.3

[1] Daily consumption per pig approximately 1.95 pounds.

In series D, groups 1 and 2, the controls fed the unheated diets, grew normally but showed a seemingly high incidence of lameness, particularly group 1. The feeding of the unsupplemented heat-treated diets resulted in lameness and incoordination in varying stages in all 18 animals fed. Growth was also retarded in all pigs and in fact stopped in group 4. This group was on test for an average of 78 days. One animal died and others appeared near death as the experiment closed.

The supplements used stimulated growth in varying degrees. Dried pork liver, fed at a level of 3 percent, was very effective, and the commercial dried whey product, fed with the simple diet at a level of 7.7 percent, produced nearly as good growth. The whey concentrate, which was fed at a level approximating 210 cc. of fresh whey to 85 gm. of heated diet, was insufficient to prevent lameness completely although none of the three affected animals showed advanced incoordination. Of the five supplements used, wheat-germ oil was entirely ineffective in prevention of lameness, whereas the corn-gluten meal, liver, rice bran, and whey products were all moderately effective at the levels fed. The striking results obtained with the heated diet in the production of lameness, together with the protection afforded by these supplements when added to the heated diet, leave little doubt of the nutritional origin of the disease.

CURATIVE TESTS

In series E, the diets fed to the three groups of affected animals that had been segregated from their litter or lot mates failed to cure the lameness and incoordination. The results are shown in table 2. Although there was a minor improvement in the average scores of groups 1 and 3, the average of all three groups shows no improvement. The 5 pigs in group 2, which received dried skim milk, showed a decided increase in the severity of the disease. The failure of the curative tests may be due to the limited degree to which regeneration of nerve tissue can take place in the animal body.

RAT-FEEDING EXPERIMENTS

The rat-feeding experiments, the results of which are given in table 3, showed that the growth of rats was retarded by the heat treatment of the diets almost as much as it was in the pigs. The simple unheated diet, as used in pig series D, group 1, produced significantly poorer growth than the stock diet. In turn, the animals on the heated simple diet were retarded in growth and required more feed per unit of gain than those on the unheated diet.

The lack of a marked response from the addition of 5 percent of casein, extracted with water and dilute alcohol, indicates that quality of protein was not the limiting factor. Alfalfa-leaf meal fed at a 5-percent level, wheat germ at 10 percent, and rice bran at 5 percent appeared to be more effective supplements than the casein. When the daily allowance of rice bran was 1 gm. per day, or approximately 8 percent of the total food intake, the growth rate was nearly as great as when the unheated diet was fed, and was greater than for the wheat-germ supplement, of which the daily intake also averaged 1 gm., although constituting 10 percent of the diet. In the feeding of fractions of the rice bran, it was found that the filtrate fraction remaining

after the thiamin, riboflavin, and vitamin B_6 had presumably been removed by adsorption treatments was the most effective preparation of the series. Neither thiamin nor riboflavin preparations were of material benefit, thus supporting the findings with the rice-bran absorbate preparation that neither of these vitamins was a limiting factor in growth.

TABLE 3.—*Results of rat-feeding experiments on the heated pig diets of series D with various supplements*

Rat group No.	Rats used in experiments	Diet fed	Average gain in 12 weeks	Feed per gram of gain
	Number		*Grams*	*Grams*
1	8	Simple, unheated (control)	144.9	7.15
2	8	Stock, unheated (control)	185.1	6.55
3	14	Simple, heated	68.7	9.92
		Simple, heated, supplemented by—		
4	6	Casein, 5 percent	86.3	7.58
5	6	Alfalfa-leaf meal, 5 percent	100.2	7.37
6	6	Linseed meal, 5 percent	89.7	8.29
7	6	Wheat germ, 10 percent	96.2	8.49
8	4	Rice bran, 5 percent	98.0	7.96
9	4	Rice bran, 8 percent	138.3	7.22
10	4	Rice bran, absorbate	96.0	8.03
11	4	Rice bran, eluate	86.5	9.92
12	4	Rice bran, filtrate	135.5	7.75
13	6	Liver extract	164.7	6.33
14	6	Whey concentrate	145.8	6.45
15	6	Thiamin, 30 micrograms per week	80.3	8.71
16	6	Riboflavin, 105 micrograms per week	76.0	9.19

Liver, and to a less extent the whey concentrate, as prepared in the Bureau of Dairy Industry, were effective in promoting better growth than the unheated diet. The results as a whole appear to support the data presented by various workers that a factor of factors found in the so-called filtrate fraction obtained in the separation of the vitamin B complex are destroyed by prolonged dry-heat treatment.

HISTOLOGICAL STUDIES OF TISSUES OF PIGS

Histological examinations of tissues of pigs showed that a degeneration of the myelin sheaths in certain peripheral nerves and the spinal cord was probably a primary factor in the development of the locomotor symptoms evidenced in lameness and incoordination. As the work progressed, the similarity of the lesions to those reported by the Iowa investigators (*2*) and to those of Wintrobe and coworkers (*8*) became evident.

The nature of the degenerative changes in the myelin sheath of nerve fibers of the sciatic nerve and the spinal cord, as shown by the Marchi procedure, is illustrated in plates 1 and 2. Varying degrees of degeneration were found, sometimes involving only an occasional nerve fiber or tract and sometimes widespread areas.

The degenerative changes in the spinal cord were confined principally to the dorsal columns and dorsal nerve roots, although some demyelination was found in the ventral columns also. The dorsal columns of the cord are made up principally of ascending branches from the dorsal root fibers, and they convey posture, locomotor, and other sensations. An interruption of conduction in these tracts will lead to incoordination and finally a complete inability to walk, depending on the extent and location of the degeneration.

Table 4 presents a summary of the results of the histological examinations expressed numerically and of the outward symptoms of locomotor involvement as shown by the lameness score. The average scores for the outward symptoms are in general agreement with those for the nerve examinations. In most instances, the number of animals involved are not greatly different for the two scoring indices. Besides the animals included as having definite lesions, a number of others not included in the table had slight changes, from the normal structure, in the sciatic nerve and spinal cord, a condition which may or may not indicate early lesions. Even the unheated stock diet protected less than two-thirds of the animals. The finding of abnormalities in the pigs receiving green forage is confirmed by the histological studies on 10 animals. The data on this group indicate a condition intermediate between those on the unheated simple diet and those on the stock diet. The heated diet resulted in the greatest amount of spinal-cord degeneration, just as it did in general incoordination. The addition of dried whey in group 8 of series D prevented the extreme degeneration noted in group 3 of that series, and the higher level of whey used in group 9 was even more effective. Although the whey concentrate furnished considerable protection, it did not appear to be equal to the dried whey on an estimated liquid-whey basis. The addition of wheat-germ oil to the heated stock diet was of little if any avail in the prevention of lameness or of nerve degeneration. Rat tests showed the wheat-germ oil to be reasonably potent as a source of vitamin E, and it seems unlikely that the substances which confer vitamin E activity are involved in the nerve degeneration in question.

TABLE 4.—*Results of histological examinations on nerve tissues compared with data on incoordination*

Series	Group No.	Diet treatment or supplement	Pigs used	Incoordination data		Histological data on—			
						Sciatic nerve		Spinal cord	
				Pigs affected	Average score	Pigs with definite lesions	Average score	Pigs with definite lesions	Average score
			Number	*Number*		*Number*		*Number*	
A	4	Green forage	10	6	0.85	7	1.00	7	1.20
B	1	Control, corn-gluten meal	1	0	.00	1	.50	1	.50
B	2	Oat supplement	1	0	.00	0	.00	0	.00
B	3	Dried pork-liver supplement	1	0	.00	0	.00	0	.00
B	5	Molasses supplement	2	1	.50	1	.25	1	.25
C	2	Barley	6	1	.08	0		0	
C	3	Wheat	6	4	.50	3	.50	4	.67
D	1	Simple, unheated	15	12	1.00	11	1.43	14	1.47
D	2	Stock, unheated	16	6	.44	7	.41	7	.56
D	3	Simple, heated	12	12	2.59	12	3.17	12	3.42
D	4	Stock, heated	[1] 4	4	2.63	4	3.00	4	3.50
D	8	Simple, heated, supplemented with dried whey	6	2	.58	3	1.83	4	1.00
		Stock, heated, supplemented with—							
D	9	Dried whey	6	1	.33	2	.33	2	.50
D	10	Whey concentrate	6	2	.50	3	1.00	4	1.17
D	11	Wheat-germ oil	[1] 4	4	1.13	3	1.75	4	2.00

[1] Two additional animals died near the close of the experiment in advanced stages of paralysis, and no histological studies were made.

A comparison of the results for groups 2 and 3 of series C, which received barley and wheat respectively, with those for group 1 of series D, which received corn as the principal ingredient of the diet, showed that wheat proved little different from corn but that barley conferred a high degree of protection. Among the pigs examined, those fed barley and those fed corn-gluten meal were the only ones that showed no definite cases of nerve degeneration. A limited number of rats from the experiments already described were examined for myelin degeneration. Animals fed the heated diet with the cod-liver oil fed in separate dishes showed degeneration, and at least one of six animals showed evidences of incoordination.

DISCUSSION

From the results thus far obtained, it seems reasonably certain that the locomotor symptoms and evidences of nerve and spinal-cord degeneration that have been found are similar to those reported by other investigators. However, Eveleth and Biester (*2*) concluded that the incoordination and myelin degeneration were caused by different etiological agents since severe incoordination was not always associated with advanced myelin degeneration. In some of their experiments, the feeding of diets deficient in vitamin A resulted in development of incoordination but only mild nerve degeneration.

Wintrobe and associates (*8*) describe demyelination in the peripheral nerves, the posterior root ganglia, and the posterior (or dorsal) columns of the spinal cord. Animals showing the degeneration had manifested "a peculiar slapping gait" and "were particularly awkward in turning." In the advanced stages, the animals became less active and sat with the hind legs sprawled in unnatural positions. The animals received an artificial diet from an early age, and the evidence pointed to a dietary deficiency of one or more components of the vitamin B complex other than thiamin or riboflavin.

In the present experiments, it was possible to control the production of the disease, within limitations, by the use of natural feedstuffs and thereby clarify some of the questions raised by the other investigators. Unlike the findings of the Iowa group (*2*), the results of the present work show a rather close association of incoordination with myelin degeneration. It seems apparent that the locomotor symptoms developed following the degeneration of fibers in the nervous system.

Thus far, repair of the degenerated fibers accompanied by disappearance of the symptoms of incoordination has not been observed. However, this result does not seem to be unusual, since a highly potent protective diet was not tested on the affected animals and regeneration of nerve tissue in the animal body is limited.

The finding that diets of the type commonly used in hog feeding, when subjected to heat treatment, will produce the disease has made it possible to study the problem in greater detail and with more certainty than formerly. There seems to be little doubt that the symptoms in live animals and myelin degeneration found histologically are similar in animals on heated diets and animals frequently affected on so-called normal diets. Heating of the diets undoubtedly destroyed or inactivated a nutritive factor or factors that are in some way related directly or indirectly to the nutrition of nerve fibers. Failure of animals to grow normally on the heated diets suggests that

more than one nutritive factor may be destroyed by heating. Observations on the growth of lame animals fed on unheated diets, including the stock or record-of-performance diet, as compared with normal animals under the same conditions, indicate that lame animals are frequently but not necessarily slower in rate of growth. Accordingly it is possible that the myelin-degeneration-preventive material is different from the growth factor destroyed by heating.

The results obtained on the heated diets have strengthened the opinion already expressed that breeding as a factor in predisposing the animals toward development of lameness is of much less concern than was at one time suspected. The absence of any serious cases of lameness in a number of litters from stock that had shown a high incidence in earlier work has contributed to this opinion.

Interference with normal bone development as a factor in the development of lameness has been eliminated by some work on the ash content of the femur bones.[3] However, the data on percentage of ash in the fat and moisture-free bone showed no relation to lameness or to diet. Another phase of the studies, namely, incidence of swine erysipelas, has been given some attention. Results of serological tests on blood serums, carried out in the laboratories of the Pathological Division of the Bureau, showed frequent positive cases of erysipelas, but there was no association of this disease with incoordination. However, lameness resulting from diseased joints due to erysipelas infections may be confused with the initial signs of incoordination due to dietary deficiency.

The identity of the lameness- and nerve-degeneration-preventive factor is still obscure. Evidently it is not present in large quantities in the usual swine feeds. Results suggest that barley and oats may be more effective preventives than corn and wheat. Liver, skim milk, whey, rice bran, and corn-gluten meal are among the feeds tested that possessed considerable value yet at the levels fed were not effective in all animals.

SUMMARY

The experimental work herein reported was carried on at the United States Department of Agriculture, Beltsville Research Center, Beltsville, Md., from the spring farrow of 1937 through the spring farrow of 1939. The pigs were generally placed on experiment at 10 to 12 weeks of age and were kept on test for 12 to 24 weeks. The disease, which frequently occurs among pigs confined in small pens with concrete floors, is characterized by incoordination in the use of the legs, abnormal posture, and lameness.

The diets used were prepared from the general supply of feeds purchased for use in swine feeding at Beltsville. The stock diet consisted of No. 2 yellow corn, digester tankage of 60-percent protein content, linseed meal, alfalfa-leaf meal made from sun-cured hay, and a mineral mixture. Various supplements or replacement feeds were used, being chosen because of known or supposed mineral, vitamin, or protein value or as contrasting feeds for those replaced in the stock diet.

Myelin degeneration of the nerves and spinal cord was demonstrated in the affected animals. The extent of degeneration was in general parallel with the severity of incoordination.

[3] Unpublished data.

When the normal diets were heated at 115°–120° C. for 30 to 40 hours, incoordination and myelin degeneration were generally produced in all animals. Heating greatly increased the incidence and severity of the disease. The incidence of the disease was high on stock diets commonly used for growing and fattening hogs.

Supplemental or replacement feeds incorporated into the basic diets, including the heated diets, gave favorable results in some cases although complete protection was infrequent. Of the various feed materials investigated, liver, concentrated milk products, barley, and oats afforded the greatest protection.

Animals that did not receive a mineral supplement in the diet tended to show a greater degree of lameness than those receiving such a supplement.

The results indicate a deficiency disease of nutritional origin. The dietary factor appears to be present in variable or insufficient quantities in many of the feeds commonly used in hog feeding and is destroyed or inactivated by dry-heat treatment.

LITERATURE CITED

(1) Ellis, N. R., Craft, W. A., and Zeller, J. H.
1936. A preliminary study of variations in free-choice intake of the components of a standard ration and breeding as possible factors in the occurrence of lameness in pigs. Amer. Soc. Anim. Prod. Proc. 29: 77–81.

(2) Eveleth, D. F., and Biester, H. E.
1937. The significance of myelin sheath degeneration and its relation to incoordination. Amer. Jour. Path. 13: 257–266, illus.

(3) Hogan, A. G., Johnson, S. R., and Gahley, E. G.
1938. Ration of sow and response of suckling pigs. Amer. Soc. Anim. Prod. Proc. 31: 112–117.

(4) Hughes, E. H.
1939. The role of riboflavin and other factors of the vitamin-B complex in the nutrition of the pig. Jour. Nutr. 17: 527–533, illus.

(5) Jukes, Thomas H.
1939. Pantothenic acid and the filtrate (chick anti-dermatitis) factor. Amer. Chem. Soc. Jour. 61: 975–976.

(6) Van Etten, C., Ellis, N. R., and Madsen, L. L.
1940. Studies on the thiamin requirement of young swine. Jour. Nutr. 20; 607–625, illus.

(7) Wehrbein, Heinrich.
1916. Paralysis of pigs. Amer. Vet. Med. Assoc. Jour. (n. s. 2) 49: [238]–244, illus.

(8) Wintrobe, Maxwell W., Mitchell, David M., and Kolb, Lawrence C.
1938. Sensory neuron degeneration in vitamin deficiency . . . Jour. Expt. Med. 68: 207–220, illus.

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